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Organic acids composition of *Cydonia oblonga* Miller leaf

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ABSTRACT

Organic acid profiles of 36 *Cydonia oblonga* Miller leaf samples, from three different geographical origins of northern (Bragança and Carrazeda de Ansiães) and central Portugal (Covilhã), harvested in three collection months (June, August and October of 2006), were determined by HPLC/UV (214 nm). Quince leaves presented a common organic acid profile, composed of six constituents: oxalic, citric, malic, quinic, shikimic and fumaric acids. *C. oblonga* leaves total organic acid content varied from 1.6 to 25.8 g/kg dry matter (mean value of 10.5 g/kg dry matter). Quinic acid was the major compound (72.2%), followed by citric acid (13.6%).

Significant differences were found in malic and quinic acids relative abundances and total organic acid contents according to collection time, which indicates a possible use of these compounds as maturity markers.

Between June and August seems to be the best period to harvest quince leaves for preparation of decoctions or infusions, since organic acids total content is higher in this season.

1. Introduction

Several studies have proved that significant health risks and benefits are associated with dietary food choice (Wildman, 2001; Fattouch et al., 2007). Mediterranean diets are characterized by abundant intakes of plant foods, such as fruits, vegetables, nuts, seeds and wild plants. Biomolecules in these plants have attracted a great deal of attention, mainly concentrated on their role in preventing diseases, such as cancers and cardio- and cerebrovascular disorders (Guthrie & Kurowska, 2001). This association is often attributed to the antioxidants present in these food products, such as phenolic compounds (phenolic acids and flavonoids), vitamin E, carotenoids, L-ascorbic acid and other organic acids, which prevent free radical damage (du Toit, Volsteedt & Apostolides, 2001; Silva, Andrade, Valentão et al., 2004).

Interest in edible plants as sources of natural bioactive compounds prompted our research group to investigate the chemical composition of quince fruit and its derivatives (Andrade, Carvalho, Seabra, & Ferreira, 1998; Ferreres, Silva, Andrade, Seabra, & Ferreira, 2003; Silva, Andrade, Ferreres et al., 2002; Silva, Andrade, Gonalves et al., 2004; Silva, Andrade, Martins, Seabra, & Ferreira, 2006; Silva, Andrade, Mendes, Seabra, & Ferreira, 2002; Silva et al., 2000; Silva, Andrade, Seabra, & Ferreira, 2001; Silva, Andrade, Valentão et al., 2004; Silva et al., 2000 & 2003; Silva, Andrade, Martins et al., 2005; Silva, Andrade, Seabra et al., 2005; Sousa et al., 2007), in terms of phenolics, organic acids and free amino acids (by HPLC-DAD and HPLC-DAD-MS/MS-ESI, HPLC/UV and GC-FID, respectively), as well as their antioxidant potential (Silva, Andrade, Valentão et al., 2004).

Nowadays, quince fruit is recognized as a good, cheap and important dietary source of health-promoting compounds, due to its biologically active constituents which are characterized by their antioxidant, antimicrobial and anti-ulcerative properties (Fattouch et al., 2007; García-Alonso, Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004; Hamauzu, Hisako, Takaroni, Kume, & Omanyuda, 2005; Hamauzu, Takaroni, Kume, Irie, & Hiramatsu, 2006; Silva, Valentão, Seabra, & Andrade, in press; Silva, Andrade, Valentão et al., 2004; Wang, Jia, Zhao, & e Wang, 2006; Yildirim, 2006).

Cydonia oblonga Miller leaves have been used, after decoction or infusion, in folk medicine for their sedative, antipyretic, anti-diarrheic and antitussive properties and for the treatment of various skin diseases (De Tommasi, De Simone, Pizza et al., 1996; Oliveira et al., 2007). Nevertheless, as far as we know, few phytochemical studies have been undertaken in this matrix. Some α- and β-ionol and flavonol glycosides have been isolated from quince leaves and identified by De Tommasi, De Simone, Pizza et al. (1996), De Tommasi, Piacente, De Simone, et al. (1996b) and Lutz, Schneider, and Winterhalter (2002). In addition, interest in edible plants

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as sources of natural antioxidants prompted us to study the phenolic composition of *C. oblonga* leaves (Oliveira et al., 2007). The phenolic profile was composed of nine 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*-glucoside, kaempferol-3-*O*-rutinoside and a kaempferol-3-*O*-glycoside. This study suggested that leaves from *C. oblonga* can be used as a great and cheap source of bioactive compounds and may have relevance in the prevention of diseases in which free radicals are implicated. Significant differences were found in 3-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acid contents, according to geographical origin and collection month, which indicates a possible use of these compounds as markers of samples with different geographical origins and/or physiological maturities (Oliveira et al., 2007).

Organic acids are primary metabolites, which can be found in great amounts in all plants, especially in fruits. Citric, malic and tartaric acids are commonly found in fruits and berries, while oxalic acid is present in higher amounts in green leaves. As phenolics, the organic acids may also have a protective role against various diseases due to their antioxidant properties (Silva, Andrade, Valentão et al., 2004; Valentão, Andrade, Rangel et al., 2005; Valentão, Lopes, Valente et al., 2005). For instance, ascorbic acid (vitamin C) is the most widely distributed water-soluble antioxidant in fruits and vegetables. Oxalic acid is the simplest dicarboxylic acid and its most striking chemical impact is its strong chelating ability for multivalent cations. Other carboxylic acids, such as tartaric, malic, citric, succinic and hydroxyglutaric, behave as antioxidants because they also have the ability to chelate metals. They are, therefore, classified as "preventive" or synergistic (Seabra et al., 2006).

Recently, the total phenolics content of *C. oblonga* leaves was reported as much higher than that found for pulps, peels and seeds, which may indicate that this part of the tree can be much more interesting in terms of health-promoting constituents (Oliveira et al., 2007). So, in continuation of our investigation on this plant species, the work herein represents a contribution to the knowledge of quince leaves' composition, concerning their organic acid profile. Additionally, we studied the possible influence of geographical origin and collection month, on the organic acids content. In this plant species, this is the first time that organic acid composition has been evaluated during the collection period.

2. Materials and methods

2.1. Samples

Thirty-six healthy quince leaves samples 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 – at 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%.

2.2. Standards

The standards were from Sigma (St. Louis, MO, USA) and from Extrasynthése (Genay, France). Methanol and hydrochloric acids were obtained from Merck (Darmstadt, Germany) and sulphuric acid were from Pronalab (Lisboa, Portugal). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Solid-phase extraction (SPE) columns

The Chromabond C18 SPE columns (70 ml/10,000 mg) were purchased from Macherey-Nagel (Duren, Germany).

2.4. Extraction of organic acids

Extraction was achieved as previously reported (Silva, Andrade, Mendes et al., 2002; Silva, Andrade, Valentão et al., 2004; Silva, Andrade, Gonçalves et al., 2004; Silva, Andrade, Martins et al., 2005; Silva, Andrade, Seabra et al., 2005 & 2006), but with some modifications. Briefly, each dried sample (ca. 0.5 g) was thoroughly mixed with methanol (10 × 25 ml), at 40 °C. The methanolic extract was filtered, concentrated to dryness, under reduced pressure (40 °C), and redissolved in acid water (pH 2 with HCl) (ca. 25 ml). The aqueous solution obtained was passed through a SPE C18 column, previously conditioned with 30 ml of methanol and 70 ml of acid water (pH 2 with HCl). The aqueous extract was evaporated to dryness under reduced pressure (40 °C), redissolved in sulphuric acid 0.01 N (2 ml) and 20 μl were analysed by HPLC.

2.5. HPLC/UV system

Separation was achieved as reported previously (Silva, Andrade, Mendes et al., 2002; Silva, Andrade, Valentão et al., 2004; Silva, Andrade, Gonçalves et al., 2004; Silva, Andrade, Martins et al., 2005; Silva, Andrade, Seabra et al., 2005 & 2006; Valentão, Andrade, Rangel et al., 2005; Valentão, Lopes, Valente et al., 2005,), with an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel® Ion 300 OA (300 × 7.7 mm), in conjunction with a column heating device at 30 °C. Elution was carried out at a solvent flow rate of 0.2 ml/min, isocratically, with 0.01 N sulphuric acid as the mobile phase. Detection was performed with a Gilson UV detector at 214 nm.

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

Organic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.

2.6. 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 the Tukey LSD test. The level of significance was set at $p \leqslant 0.05$.

3. Results and discussion

Results from this study indicate that *C. Oblonga* leaf organic acid profile (qualitative and quantitative), is different from that of quince fruit (pulp, peel and seed) and jam (Silva, Andrade, Mendes et al., 2002; Silva, Andrade, Valentão et al., 2004; Silva, Andrade, Gonçalves et al., 2004; Silva, Andrade, Martins et al., 2005; Silva, Andrade, Seabra et al., 2005 & 2006).

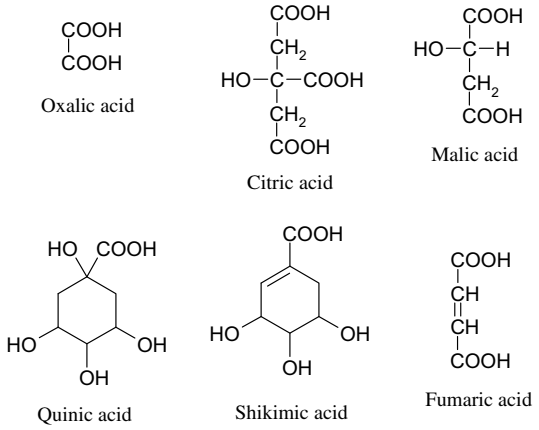

Fig. 1. Organic acids of quince leaves.

Cydonia oblonga leaf presented a chemical profile composed of six organic acids: oxalic, citric, malic, quinic, shikimic and fumaric acids (Figs. 1 and 2). Quince leaves were rich in quinic acid (72.2%), had medium values of citric, malic and oxalic acids (mean values of 13.6%, 7.6% and 6.1%, respectively) and very small proportions of shikimic and fumaric acids (<1%) (Table 2).

The total organic acids content varied from 1.6 to 25.8 g/kg dry matter (mean value of 10.5 g/kg dry matter). Considering the drying yields of quince leaves samples (about 50%), the total content (between 0.8 and 13 g/kg fresh matter; mean value of 5 g/kg fresh matter) was lower than those found for pulps (varying from 2 to 17 g/kg fresh matter) and peels (between 3 and 16 g/kg fresh matter) and higher than that of the seeds (varying from 0.5 to 0.8 g/kg fresh matter) (Silva, Andrade, Martins et al., 2005; Silva, Andrade, Seabra et al., 2005).

When compared to quince pulps and peels (Silva, Andrade, Martins et al., 2005, Silva, Andrade, Seabra et al., 2005), leaves presented a characteristic qualitative and quantitative organic acid profile, in which ascorbic acid was absent; the malic plus quinic acids relative content was lower and oxalic, citric, shikimic and fumaric acids relative percentages were higher. Considering quince seeds profile (Silva, Andrade, Seabra et al., 2005), the differences were the presence of oxalic acid, the lower citric and fumaric acid relative contents, the higher malic plus quinic and shikimic acid percentages and absence of ascorbic acid.

It is well known that leaves protect fruits from UV radiation and have an important role in the photosynthesis process, since they are able to convert light energy into chemical energy (glucose and ATP), by scavenging CO_2 and producing O_2. Later, glucose can be used to produce several metabolites (polysaccharides, amino acids, organic acids, phenolic compounds) according to plant needs. So, it is from leaves that nutrient distribution is carried

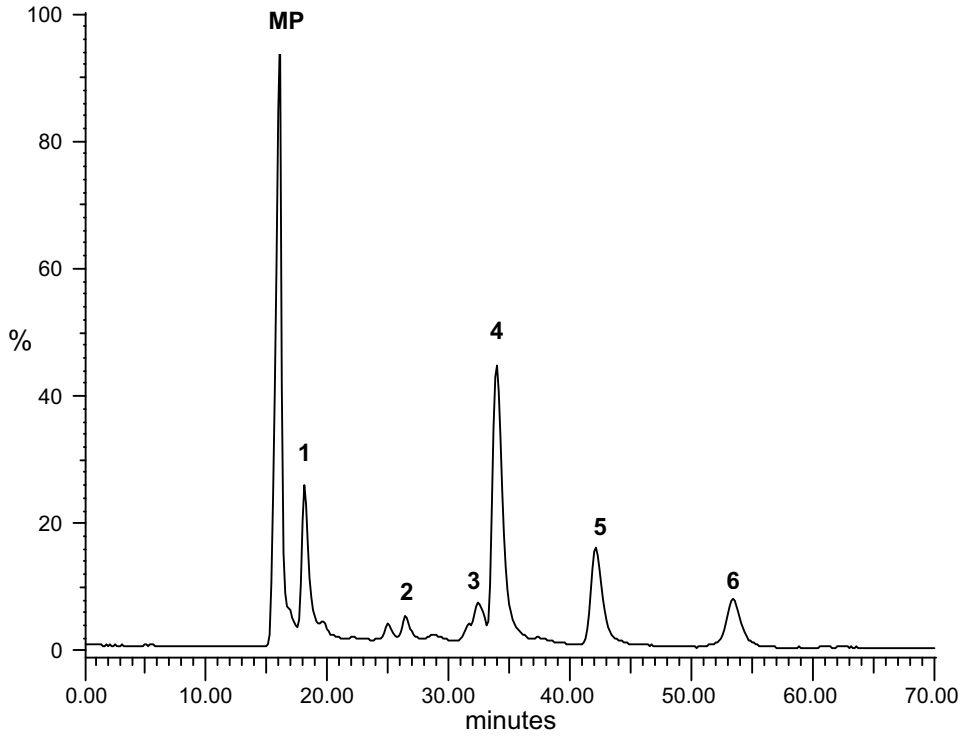

Fig. 2. HPLC organic acid profile of quince leaves. Detection at 214 nm. Peaks: (MP) mobile phase; (1) oxalic acid; (2) citric acid; (3) malic acid; (4) quinic acid; (5) shikimic acid; (6) fumaric acid.

out, to all parts of the tree. In quince leaves, ascorbic acid was not present and the organic acids total content was lower than that found for pulps and peels, but phenolics total content was much higher (Oliveira et al., 2007). Phenolics from quince leaves, namely flavonol derivatives, may have an important role, protecting cells (membranes, chlorophylls and other fragile organelles) from the

Table 2
Organic acids composition of quince leaves samples[a]

Sample	Organic Acids (%)						
	Oxalic acid	Citric acid	Malic acid	Quinic acid	Shikimic acid	Fumaric acid	∑ (g/kg)
1	3.90 ± 0.07	7.90 ± 0.42	7.61 ± 0.04	80.1 ± 1.51	0.98 ± 0.01	0.11 ± 0.01	16.9
2	3.75 ± 0.04	8.26 ± 0.10	6.88 ± 0.06	80.8 ± 0.62	0.35 ± 0.01	0.01 ± 0.00	22.1
3	5.14 ± 0.23	39.2 ± 0.27	4.85 ± 0.03	50.3 ± 0.38	0.52 ± 0.01	nd	4.93
4	2.74 ± 0.03	4.69 ± 0.19	6.21 ± 0.03	86.0 ± 0.67	0.24 ± 0.00	0.10 ± 0.01	16.6
5	12.0 ± 0.56	4.28 ± 0.04	7.59 ± 0.02	75.8 ± 0.37	0.29 ± 0.01	0.08 ± 0.01	15.4
6	10.5 ± 0.14	6.36 ± 0.24	12.5 ± 0.52	70.3 ± 0.26	0.21 ± 0.01	0.08 ± 0.01	6.85
7	2.83 ± 0.28	4.29 ± 0.08	8.55 ± 0.01	83.4 ± 0.08	0.23 ± 0.01	0.07 ± 0.01	16.2
8	4.09 ± 0.01	17.8 ± 0.46	14.5 ± 0.05	63.3 ± 2.00	0.28 ± 0.01	0.06 ± 0.01	17.9
9	5.45 ± 0.07	12.9 ± 0.09	19.1 ± 0.03	62.0 ± 0.62	0.52 ± 0.02	nd	2.52
10	1.86 ± 0.10	31.0 ± 0.45	2.58 ± 0.17	64.2 ± 0.28	0.35 ± 0.01	nd	10.4
11	10.5 ± 0.27	11.9 ± 0.37	11.0 ± 0.32	66.5 ± 1.23	0.04 ± 0.01	0.08 ± 0.01	14.0
12	11.4 ± 0.56	nd	14.8 ± 0.46	72.90 ± 0.19	0.95 ± 0.03	nd	1.64
13	1.05 ± 0.01	1.77 ± 0.13	6.44 ± 0.07	90.4 ± 0.73	0.19 ± 0.00	0.11 ± 0.00	13.8
14	2.58 ± 0.03	6.64 ± 0.08	8.28 ± 0.09	81.8 ± 1.45	0.50 ± 0.01	0.19 ± 0.01	12.9
15	4.59 ± 0.05	20.7 ± 1.18	11.2 ± 0.05	61.0 ± 2.62	2.40 ± 0.12	0.03 ± 0.01	1.90
16	9.41 ± 0.47	4.29 ± 0.24	5.70 ± 0.24	80.4 ± 0.03	0.23 ± 0.01	nd	14.6
17	18.2 ± 0.63	4.99 ± 0.14	7.66 ± 0.03	68.8 ± 0.26	0.29 ± 0.01	0.08 ± 0.01	11.3
18	4.63 ± 0.24	12.2 ± 1.15	10.9 ± 0.10	70.9 ± 0.97	1.36 ± 0.01	0.02 ± 0.01	3.05
19	0.46 ± 0.01	26.5 ± 0.02	3.19 ± 0.08	69.4 ± 0.11	0.42 ± 0.01	nd	4.35
20	7.83 ± 0.10	7.94 ± 0.31	9.45 ± 0.23	74.2 ± 2.85	0.44 ± 0.02	0.14 ± 0.01	16.8
21	5.64 ± 0.11	27.2 ± 0.48	12.6 ± 0.38	54.0 ± 1.51	0.57 ± 0.01	nd	2.45
22	20.8 ± 0.04	26.2 ± 0.09	2.97 ± 0.01	48.9 ± 0.39	1.14 ± 0.01	nd	9.88
23	3.97 ± 0.02	16.5 ± 0.13	8.38 ± 0.09	70.6 ± 0.89	0.55 ± 0.01	nd	3.28
24	8.84 ± 0.16	15.1 ± 0.41	17.2 ± 0.01	58.2 ± 0.29	0.58 ± 0.02	0.02 ± 0.01	2.06
25	2.53 ± 0.07	5.20 ± 0.03	0.93 ± 0.04	91.0 ± 1.91	0.38 ± 0.01	nd	12.7
26	5.30 ± 0.04	15.6 ± 0.01	5.70 ± 0.06	72.5 ± 0.29	0.90 ± 0.01	nd	6.53
27	14.0 ± 0.20	nd	2.17 ± 0.02	83.8 ± 1.72	0.04 ± 0.01	nd	1.88
28	9.82 ± 0.02	7.61 ± 0.45	3.42 ± 0.03	78.8 ± 0.04	0.25 ± 0.01	0.01 ± 0.01	25.8
29	3.18 ± 0.01	29.7 ± 1.26	2.63 ± 0.10	64.2 ± 2.41	0.37 ± 0.02	nd	9.72
30	0.60 ± 0.04	9.30 ± 0.05	3.78 ± 0.08	85.9 ± 1.79	0.38 ± 0.01	0.07 ± 0.00	6.32
31	1.07 ± 0.03	12.1 ± 0.07	6.95 ± 0.14	79.5 ± 0.12	0.37 ± 0.01	0.11 ± 0.01	18.9
32	3.04 ± 0.30	18.2 ± 0.15	7.62 ± 0.72	70.7 ± 4.42	0.47 ± 0.01	nd	7.22
33	8.28 ± 0.14	4.92 ± 0.11	7.62 ± 0.15	79.2 ± 1.92	nd	nd	1.85
34	3.93 ± 0.07	3.15 ± 0.01	3.20 ± 0.17	89.3 ± 0.86	0.37 ± 0.01	0.06 ± 0.01	22.5
35	3.12 ± 0.02	32.0 ± 0.39	2.22 ± 0.04	62.2 ± 0.40	0.38 ± 0.03	nd	12.8
36	3.13 ± 0.13	32.8 ± 0.01	6.83 ± 0.13	56.6 ± 1.82	0.54 ± 0.01	0.09 ± 0.01	8.89
Mean	6.12	13.6	7.59	72.2	0.50	0.04	10.5
Max	20.8	39.2	19.1	91.0	2.40	0.19	25.8
Min	0.46	nd	0.93	48.9	nd	nd	1.64
SD	4.81	10.66	4.40	11.3	0.44	0.05	6.77

[a] Values (as percentages) are expressed as mean ± standard deviation of three assays for each sample. Abreviations: nd, not detected; SD, standard deviation; ∑, sum of the determined organic acids.

Table 3
Organic acids composition of quince leaves, according to collection month (means ± SD)

Month	Organic acids (%)						
	Oxalic acid	Citric Acid	Malic acid	Quinic acid	Shikimic acid	Fumaric acid	∑ (g/kg)
Bragança							
June	2.83 ± 0.84 a	12.0 ± 12.79 a	6.24 ± 2.62 a	78.4 ± 9.78 a	0.30 ± 0.08 a	0.07 ± 0.05 a	15.0 ± 3.07 a
August	7.59 ± 4.28 a	10.6 ± 5.76 a	9.99 ± 3.49 a	71.6 ± 8.10 a	0.24 ± 0.14 a	0.06 ± 0.03 a	17.3 ± 3.56 a
October	8.13 ± 3.29 a	14.6 ± 17.2 a	12.8 ± 5.96 a	63.9 ± 10.2 a	0.55 ± 0.30 a	0.02 ± 0.04 a	3.99 ± 2.36b
Carrazeda de Ansiães							
June	7.93 ± 9.50 a	14.7 ± 13.5 a	4.58 ± 1.75b	72.3 ± 17.81 a	0.50 ± 0.44 a	0.03 ± 0.06 a	10.7 ± 4.67 a,b
August	8.14 ± 7.06 a	9.01 ± 5.11 a	8.44 ± 2.57 b	73.9 ± 5.76 a	0.45 ± 0.11 a	0.10 ± 0.08 a	11.1 ± 5.67 a
October	5.93 ± 2.00 a	18.8 ± 6.61 a	13.0 ± 2.92 a	61.0 ± 7.16 a	1.23 ± 0.86 a	0.02 ± 0.01 a	2.37 ± 0.51 b
Covilhã							
June	4.34 ± 3.84 a	7.00 ± 3.83 a	3.63 ± 2.49 a	84.7 ± 6.36 a	0.34 ± 0.06 a	0.05 ± 0.05 a	20.0 ± 5.64 a
August	3.66 ± 1.09 a	23.9 ± 8.19 a	4.54 ± 2.57 a	67.4 ± 4.98 a	0.53 ± 0.25 a	0.00 ± 0.00 a	9.07 ± 2.84 b
October	6.51 ± 5.94 a	11.8 ± 11.5 a	5.10 ± 2.56 a	76.4 ± 13.4 a	0.24 ± 0.26 a	0.04 ± 0.05 a	4.74 ± 3.48 b
All sites combined							
June	5.03 ± 5.82 a	11.2 ± 10.5 a	4.81 ± 2.38 b	78.4 ± 12.3 a	0.38 ± 0.25 a	0.05 ± 0.05 a	15.2 ± 5.75 a
August	6.46 ± 4.82 a	14.5 ± 9.11 a	7.66 ± 3.32 a,b	70.9 ± 6.44 a,b	0.41 ± 0.20 a	0.05 ± 0.06 a	12.5 ± 5.29 a
October	6.85 ± 3.82 a	15.1 ± 12.6 a	10.3 ± 5.34 a	67.1 ± 11.8 b	0.67 ± 0.66 a	0.03 ± 0.03 a	3.70 ± 2.44 b

In the same column, means with different letters are significantly different ($p \leqslant 0.05$).

damage caused by UV radiation. Probably, leaves produce these secondary compounds because they are more efficient antioxidants than are organic acids. This is in agreement with previous studies performed by our research group, where we have reported quince fruit (pulp, peel and seed) and jam methanolic extract scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Silva, Andrade, Valentão et al., 2004). Methanolic extracts were fractionated into phenolic and organic acid fractions. The results indicated that IC_{50} of the total methanolic extracts was only correlated with the caffeoylquinic acid total contents and the phenolic fraction always exhibited a stronger antioxidant activity than did the whole methanolic extract.

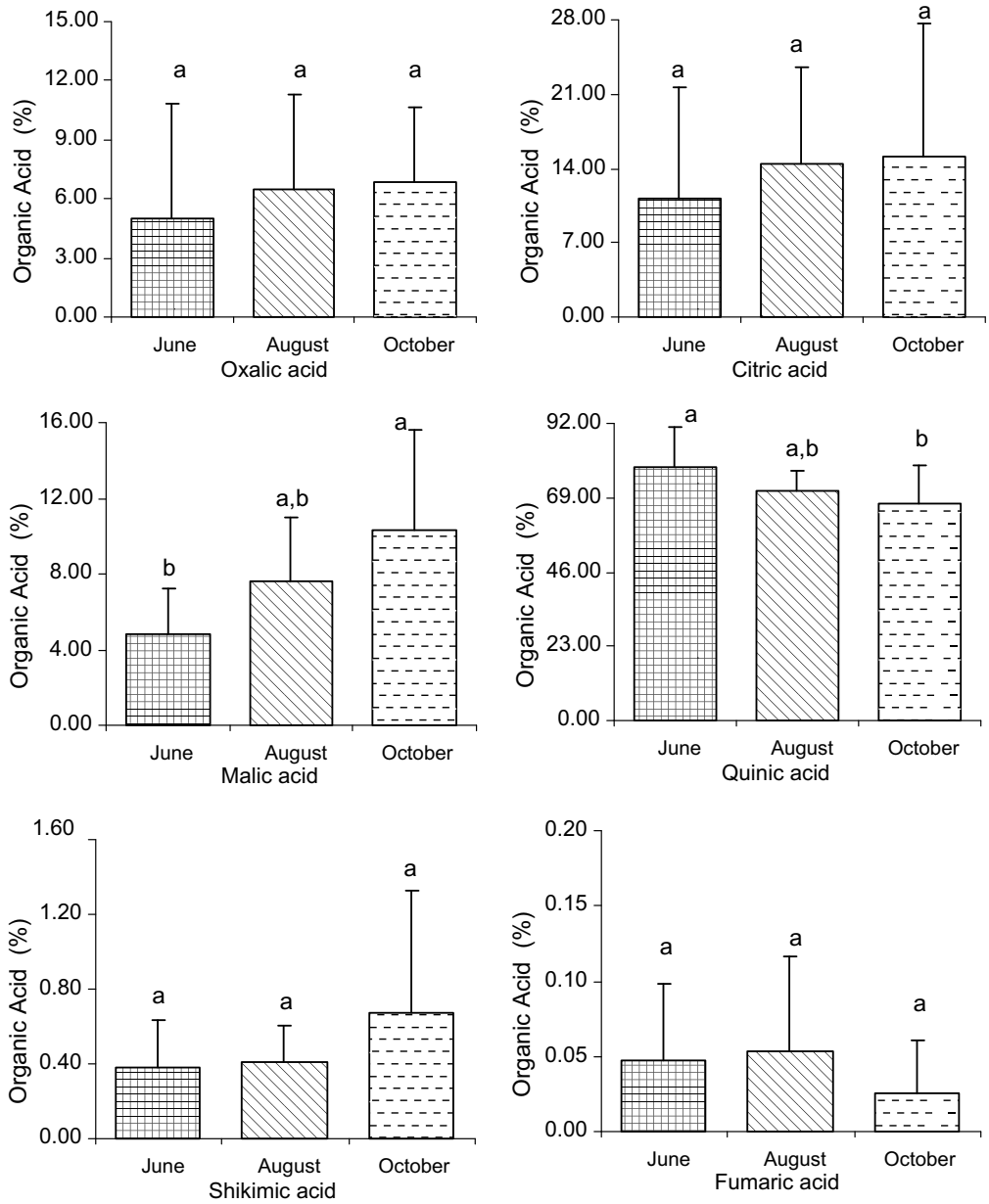

Fig. 3. Quantitative organic acid profile of quince leaves' samples (%), according to collection month (considering samples from all geographical origins). For each compound, means with different letters are significantly different ($p \leqslant 0.05$).

Table 4
Organic acids composition of quince leaves, according to geographical origin (means ± SD)

Geographical origin	Organic acids (%)						
	Oxalic acid	Citric acid	Malic acid	Quinic acid	Shikimic acid	Fumaric acid	∑ (g/kg)
Bragança	6.18 ± 3.78 a	12.4 ± 11.73 a	9.68 ± 4.77 a	71.3 ± 10.5 a	0.36 ± 0.23 a	0.05 ± 0.04 a	12.1 ± 6.68 a
Carrazeda de Ansiães	7.33 ± 6.36 a	14.2 ± 9.31 a	8.67 ± 4.03 a	69.1 ± 12.1 a	0.72 ± 0.63 a	0.05 ± 0.07 a	8.02 ± 5.68 a
Covilhã	4.84 ± 3.93 a	14.2 ± 11.61 a	4.42 ± 2.38 b	76.1 ± 11.0 a	0.37 ± 0.23 a	0.03 ± 0.04 a	11.3 ± 7.68 a

In the same column, means with different letters are significantly different ($p \leqslant 0.05$).

Significant differences ($p \leqslant 0.05$) were found among samples harvested in the three different months (concerning malic and quinic acid percentages and total organic acid contents). Samples collected in October presented a lower organic acids total content, considering each geographical origin or all of them at the same time (Table 3; Fig. 3). Generally, the amount of these acids decreased during harvesting time, which can be related to the high temperature verified during summer time, that implies physiological adaptations of plants. Malic and quinic acid contents also varied according to the collection date ($p \leqslant 0.05$). Leaves' composition, in terms of these two acids, was inverse. Samples which presented high malic acid contents exhibited low quinic acid abundance and vice-versa.

Malic acid content of samples collected in Covilhã (Central Portugal) was significantly lower than those from Bragança and Carrazeda de Ansiães (northern Portugal) samples ($p \leqslant 0.05$) (Table 4; Fig. 4).

As far as we know, few chemical studies concerning leaves' organic acid profiles have been developed. Before kombucha tea fermentation (green and black tea leaves), the main organic acid is D-glucuronic acid. Nevertheless, acetic, lactic and citric acids are also found after fermentation and their contents are significant changed during fermentation time (Jayabalan, Marimuthu, & Swaminathan, 2007). Seven organic acids – aconitic, citric, ascorbic, malic, quinic, shikimic and fumaric acids – were identified and quantified in *Brassica oleracea* L. var. *costata* DC internal leaves and citric acid was the main compound (Ferreres et al., 2006) while, in *B. oleracea* var. *costata* external leaves, only citric, ascorbic, malic, shikimic and fumaric acids were found, malic acid being the major one (Vrchovská et al., 2006). *Rumex induratus* leaves were characterized by great oxalic acid concentrations (Ferreres et al., 2006). So, this study suggests that quince leaf has a qualitative and quantitative organic acid profile very distinct from those of other species. As

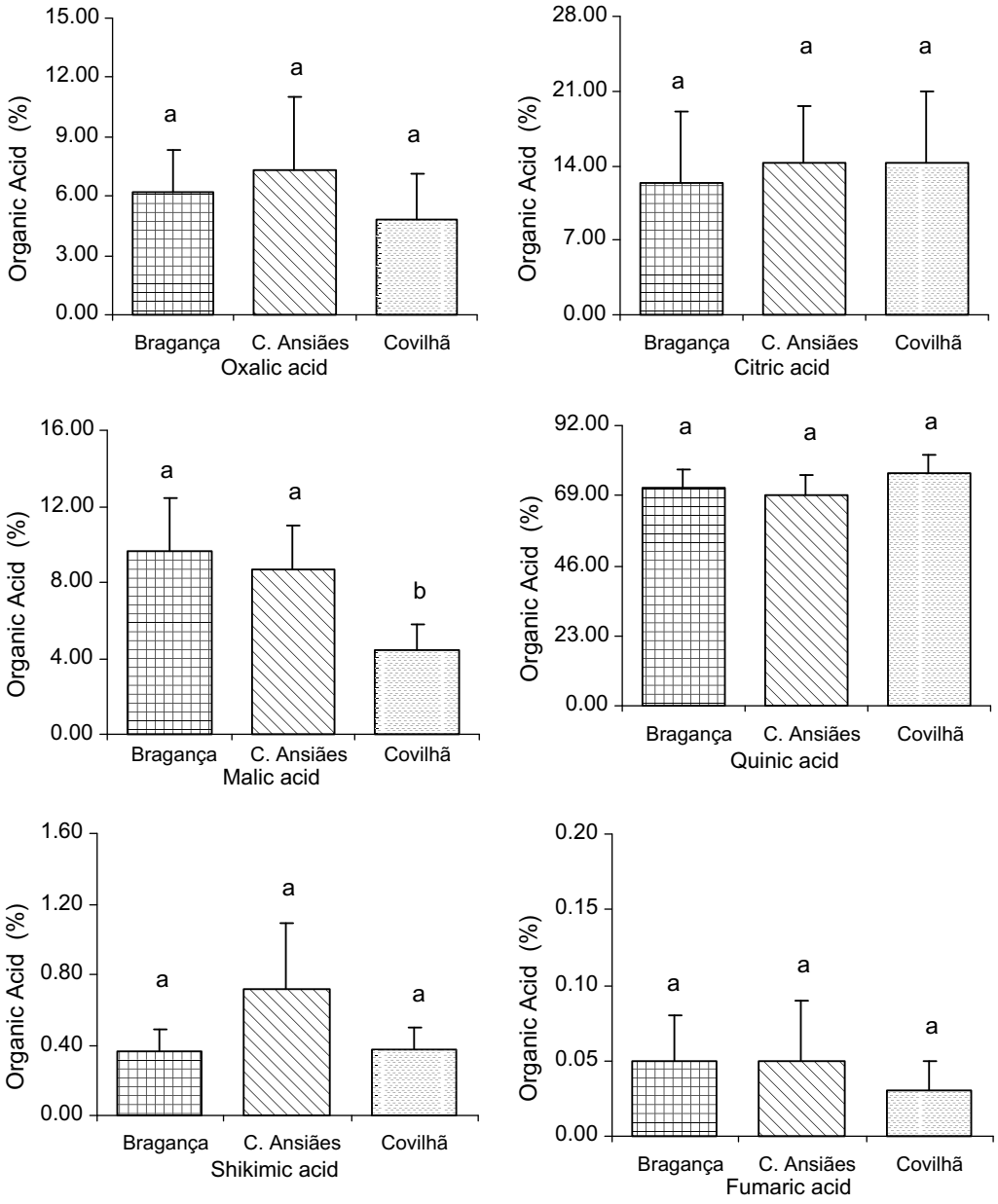

Fig. 4. Quantitative organic acid profile of quince leaves' samples (%), according to geographical origin. For each compound, means with different letters are significantly different ($p \leqslant 0.05$).

far as we know, nothing about organic acid composition of leaves from trees has been reported.

4. Conclusion

Significant differences were observed in malic and quinic acid contents of *C. oblonga* leaves, according to harvesting month, suggesting a possible use of these organic acids as maturity markers.

Between June and August seems to be the best time to collect quince leaves for preparation of decoctions or infusions, since organic acid total contents were higher in this period. Probably, this difference was due to edapho-climatic factors, mainly solar exposure.

Considering quince leaves' composition, in terms of organic acids and phenolic compounds, we intend to progress our studies, in order to evaluate their antioxidant and antimicrobial potential and bioactive compounds in comparison to quince fruit.

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