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COMPOSITION OF QUINCE (*CYDONIA OBLONGA* MILLER) SEEDS: PHENOLICS, ORGANIC ACIDS AND FREE AMINO ACIDS

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Phenolic compounds, organic acids and free amino acids of quince seeds were determined by HPLC/DAD, HPLC/UV and GC/FID, respectively. Quince seeds presented a phenolic profile composed of 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic and 3,5-dicaffeoylquinic acids, lucenin-2, vicenin-2, stellarin-2, isoschaftoside, schaftoside, 6-*C*-pentosyl-8-*C*-glucosyl chrysoeriol and 6-*C*-glucosyl-8-*C*-pentosyl chrysoeriol. Six identified organic acids constituted the organic acid profile of quince seeds: citric, ascorbic, malic, quinic, shikimic and fumaric acids. The free amino acid profile was composed of 21 identified free amino acids and the three most abundant were glutamic and aspartic acids and asparagine.

Keywords: *Cydonia oblonga* Miller; Quince seeds; Phenolics; Organic acids; Free amino acids

INTRODUCTION

Quince is the fruit of a deciduous tree of the Rosaceae family, *Cydonia oblonga* Miller. Due to its hardness, acidity and astringency, it is not edible fresh; nevertheless, it is often used to prepare jam.

Some chemical studies have been previously carried out for quince fruit. In 1979 and 1986, the volatile constituents of quince were analysed by GC/MS [1,2]. Through analysis of the phenolic profile, glucosides of procyanidin polymers have been identified in this fruit [3,4] and, recently, it has been possible to discriminate quince pulp and peel by the analysis of phenolics [5]. In 2002, an HPLC/UV method was developed for the determination of organic acids in quince fruit (pulp and peel) [6]. More recently,

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a GC/FID methodology was developed for the analysis of free amino acids in the same matrices [7].

As far as we know, for quince seeds, few chemical studies have been developed. Ion-trap HPLC-PAD-ESI/MS/MS has been used in a qualitative study of *C*-glycosyl flavones in these seeds [8]. As part of a continuing study of quince, the work herein represents a contribution to the composition of quince seeds, in terms of phenolic compounds, organic acids and free amino acids.

RESULTS AND DISCUSSION

Phenolic Compounds

Quince seeds presented a phenolic profile composed of 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic and 3,5-dicaffeoylquinic acids, lucenin-2 (6,8-di-*C*-glucosyl luteolin), vicenin-2 (6,8-di-*C*-glucosyl apigenin), stellarin-2 (6,8-di-*C*-glucosyl chrysoeriol), isoschaftoside (6-*C*-arabinosyl-8-*C*-glucosyl apigenin), schaftoside (6-*C*-glucosyl-8-*C*-arabinosyl apigenin), 6-*C*-pentosyl-8-*C*-glucosyl chrysoeriol and 6-*C*-glucosyl-8-*C*-pentosyl chrysoeriol (Fig. 1).

While quince pulps contained mainly caffeoylquinic acids and quince peels presented both caffeoylquinic acids and several flavonol glycosides in great amounts [5], quince seeds were characterised by the presence of caffeoylquinic acids and, specially, *C*-glycosyl flavones. Caffeoylquinic acids ranged from *ca.* 34 to 37% of the determined phenolics, with 5-*O*-caffeoylquinic acid being the most abundant (from 19 to 24%), while *C*-glycosyl flavones ranged from 63 to 66%, with isoschaftoside being the major one (*ca.* 18%). The total phenolic content was *ca.* 0.1 g/kg (Table I), which is

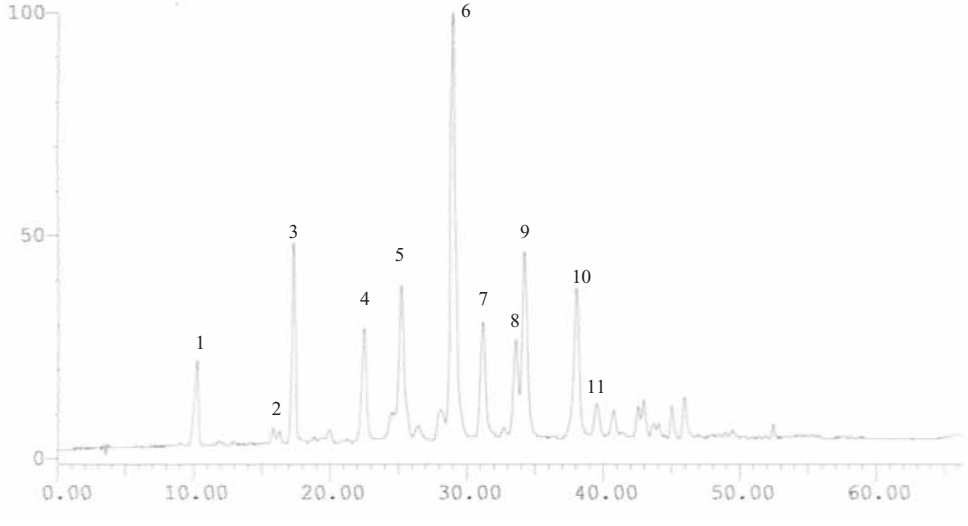

FIGURE 1 HPLC phenolic profile of quince seeds (350 nm). 1 – 3-*O*-caffeoylquinic acid; 2 – 4-*O*-caffeoylquinic acid; 3 – 5-*O*-caffeoylquinic acid; 4 – lucenin-2; 5 – vicenin-2; 6 – stellarin-2; 7 – isoschaftoside; 8 – schaftoside; 9 – 6-*C*-pentosyl-8-*C*-glucosyl chrysoeriol; 10 – 6-*C*-glucosyl-8-*C*-pentosyl chrysoeriol; 11 – 3,5-dicaffeoylquinic acid.

TABLE I Phenolic composition of quince seeds (mg/kg)

Phenolic compounds	*Samples*					
	2000		*2001*		*2002*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
3-*O*-caffeoylquinic acid	6.1	0.06	10.1	0.07	12.4	0.40
4-*O*-caffeoylquinic acid	1.4	0.08	1.1	0.03	1.7	0.01
5-*O*-caffeoylquinic acid	27.8	0.24	20.0	0.24	24.6	0.69
Lucenin-2	6.7	0.07	7.6	0.13	5.2	0.23
Vicenin-2	13.9	0.17	12.5	0.10	14.2	0.14
Stellarin-2	20.6	0.21	18.9	0.42	20.5	0.37
Isoschaftoside	10.8	0.03	8.7	0.13	10.0	0.07
Schaftoside	nq	–	6.4	0.01	7.1	0.11
6-*C*-pentosyl-8-*C*-glucosyl chrysoeriol	15.4	0.13	9.0	0.14	8.0	0.38
6-*C*-glucosyl-8-*C*-pentosyl chrysoeriol	8.2	0.26	7.7	0.14	7.7	0.43
3,5-*O*-dicaffeoylquinic acid	5.6	0.01	5.3	0.11	4.4	0.10
$\sum$	116.4		107.4		115.8	

Values are expressed as mean of the three determinations; *SD* – standard deviation; $\sum$ – sum of the determined phenolic compounds; nq – not quantified.

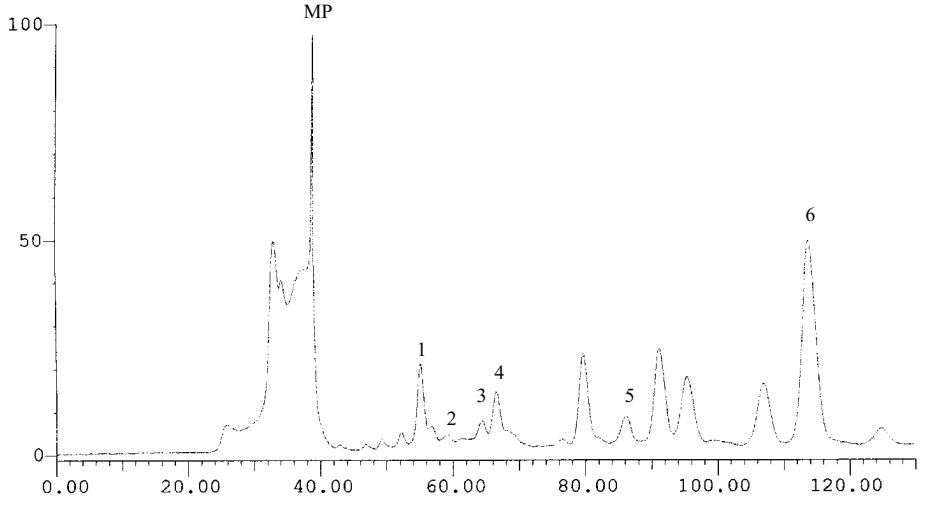

FIGURE 2 HPLC organic acid profile of quince seeds. MP – mobile phase; 1 – citric acid; 2 – ascorbic acid; 3 – malic acid; 4 – quinic acid; 5 – shikimic acid; 6 – fumaric acid.

similar to that obtained for pulps, but much lower than the one that was present in the peels [5].

Organic Acids

Seeds presented an organic acid profile composed of six identified organic acids: citric, ascorbic, malic, quinic, shikimic and fumaric acids (Fig. 2). Contrary to both quince pulp and peel [6], oxalic acid was not detected in quince seeds.

TABLE II Organic acid composition of quince seeds (mg/kg)

Organic acids	*Samples*					
	2000		*2001*		*2002*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Citric acid	213.0	10.11	164.9	4.08	93.2	1.15
Ascorbic acid	209.1	2.19	83.9	0.72	86.1	8.16
Malic + quinic acids	352.0	0.40	383.0	24.34	281.8	14.36
Shikimic acid	1.6	0.02	1.9	0.05	1.4	0.17
Fumaric acid	6.2	0.26	3.3	0.05	1.8	0.04
$\sum$	781.9		636.9		464.2	

Values are expressed as mean of the three determinations; *SD* – standard deviation; $\sum$ – sum of the determined organic acids.

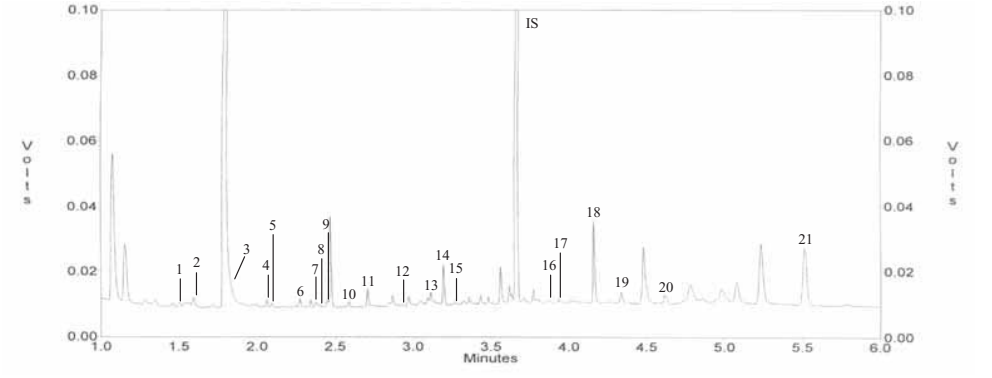

FIGURE 3 GC free amino acid profile of quince seeds. 1 – alanine; 2 – glycine; 3 – valine; 4 – leucine; 5 – isoleucine; 6 – proline; 7 – threonine; 8 – serine; 9 – glutamic acid; 10 – asparagine; 11 – aspartic acid; 12 – methionine; 13 – hydroxyproline; 14 – phenylalanine; 15 – cysteine; IS – internal standard (L-*p*-chlorophenylalanine); 16 – glutamine; 17 – ornithine; 18 – lysine; 19 – histidine; 20 – tyrosine; 21 – tryptophan.

The total organic acid content ranged from *ca.* 0.5 to 0.8 g/kg (Table II), which is much lower than the one that was obtained for quince pulp and peel collected in 2000 [6]. The sum of malic acid plus quinic acid represented 45 to 61% of the total organic acid content, while in quince pulp and peel, malic and quinic acids always constituted more than 93% [6]. Citric and ascorbic acids in seeds were also present in great percentages, the first acid ranged from 20 to 27% and the second one ranged from 13 to 27% of the determined organic acids, while in pulp and peel, these acids were present in smaller percentages (< 6%) [6]. In seeds, as previously reported for quince pulp and peel [6], shikimic and fumaric acids were present in very low amounts (< 1%).

Free Amino Acids

Samples presented a free amino acid profile composed of 21 identified free amino acids. It was not possible to quantify valine, once it coeluted with an interference compound (Fig. 3).

The sum of the 21 free amino acids ranged from *ca.* 1.3 to 1.7 mg/kg (Table III). The total free amino acids content was higher than that found in pulp and peel from fruits collected in 2000 (mean values of 0.8 and 1.0 mg/kg, respectively). As it happened with

TABLE III Free amino acids composition of quince seeds (μg/kg)

Amino acids	*Samples*					
	2000		*2001*		*2002*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Alanine	23.5	0.89	20.1	0.88	72.7	6.31
Glycine	34.6	1.31	42.5	1.48	15.8	0.16
Valine	nq	–	nq	–	nq	–
Leucine	5.4	0.13	15.0	0.60	16.4	0.41
Isoleucine	3.6	0.07	9.1	0.21	7.1	0.04
Proline	6.5	0.24	8.7	0.21	8.0	0.23
Threonine	25.4	1.11	115.0	3.24	56.7	0.37
Serine	71.3	1.54	29.7	1.15	42.8	3.40
Glutamic acid	314.2	8.92	525.3	26.51	740.5	31.84
Asparagine	106.8	4.04	230.2	4.84	191.3	6.39
Aspartic acid	470.1	8.35	201.7	5.48	367.4	11.92
Methionine	2.2	0.07	5.1	0.05	3.2	0.13
Hydroxyproline	106.5	4.96	161.8	1.70	12.3	0.77
Phenylalanine	11.1	0.28	22.9	0.56	21.5	0.04
Cysteine	4.9	0.09	2.9	0.01	2.7	0.06
Glutamine	9.5	0.43	21.2	0.26	60.0	2.29
Ornithine	2.3	0.06	6.3	0.22	1.0	0.02
Lysine	45.6	1.96	60.5	1.80	21.7	0.40
Histidine	28.8	0.99	50.6	1.43	22.9	0.02
Tyrosine	4.6	0.05	7.8	0.34	7.7	0.61
Tryptophan	68.6	4.86	47.4	1.25	49.5	3.90
$\sum$	1345.3		1583.6		1721.1	

Values are expressed as mean of the three determinations; *SD* – standard deviation; $\sum$ – sum of the determined free amino acids; nq – not quantified.

some pulp and peel (data not shown), the three major free amino acids were glutamic acid, aspartic acid and asparagine, which constituted 60 to 75% of the totality of free amino acids.

In conclusion, the phenolic profile constitutes the most useful chemical parameter for the discrimination of quince seed, pulp and peel. Seeds can be characterized by the presence of several *C*-glycosyl flavones (which are absent in pulp and peel). In what concerns to organic acid composition, in seeds, malic and quinic acids are not so predominant as in pulp and peel (citric and ascorbic acids are also present in considerable amounts). The sum of all acids is, nevertheless, much lower. The free amino acid profile does not seem to be so characteristic of this part of quince fruit.

EXPERIMENTAL

Samples

Healthy quince fruits were collected in several different locations of Northern (Amarante, Baião, Vila Real and Bragança) and Central Portugal (Viseu, Pinhel and Covilhã), in the years of 2000, 2001 and 2002. Seeds were freeze-dried using a Labconco 4.5 apparatus (Kansas City, MO). Owing to plant material scarcity, equal portions of lyophilised seeds from each geographical region were combined and pulverized.

Standards

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

Solid-phase Extraction (SPE) Columns

The ISOLUTE C18 non end-capped (NEC) SPE columns (50 μm particle size, 60 Å porosity; 10 g sorbent mass/70 mL reservoir volume) were purchased from International Sorbent Technology Ltd (Mid Glamorgan, UK). The benzenesulfonic SCX Spe-ed SPE cartridges (200 mg; 3 mL) were obtained from Applied Separations (Allentown, USA).

Extraction of Phenolic Compounds

The extraction of phenolics was achieved as previously reported [5] and included a C18 NEC SPE cleaning step.

Extraction of Organic Acids

The sample preparation was simple, involving only extraction with methanol (40°C) and filtration through a C18 NEC SPE cartridge, as reported by Silva *et al.* [6].

Extraction of Free Amino Acids

According to Silva *et al.* [7], the extraction of L-amino acids was simple, including a SCX SPE purification step.

Derivatization Procedure

The derivatization of L-amino acids was carried out as reported previously [7].

HPLC Analysis of Phenolics

The extracts were analysed on an analytical HPLC unit (Gilson), using an Spherisorb ODS2 (25.0 × 0.46 cm; 5 μm, particle size) column [5]. Detection was achieved with a Gilson DAD.

Phenolics were identified by comparison of their retention times, UV-Vis spectra in the 200–400 nm range, and MS with those obtained from standards [5,8].

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. 3- and 4-*O*-caffeoylquinic, and 3,5-dicaffeoylquinic acids were quantified as 5-*O*-caffeoylquinic acid. Luteolin, apigenin and chrysoeriol derivatives were quantified as 7-*O*-glucosyl luteolin, 7-*O*-glucosyl apigenin and chrysoeriol, respectively.

HPLC Analysis of Organic Acids

The separation was carried out as previously reported [6] with an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel® Ion 300 OA (300 × 7.7 mm). Detection was performed with an UV detector set at 214 nm.

Organic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. Malic and quinic acids were quantified together and as malic acid. The other acids were quantified as themselves.

GC Analysis of Free Amino Acids

The extracts were analysed on a Chrompack CP 9001 instrument (Chrompack, Middelburg, The Netherlands) equipped with a flame ionisation detector (FID), and an automatic liquid sampler (CP-9050, Chrompack) [7].

The amino acids were identified by their retention times and chromatographic comparison with authentic standards. Quantification was based on the internal standard method using L-*p*-chlorophenylalanine.

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