Phenolic Profile of Quince Fruit (Cydonia oblonga Miller) (Pulp and Peel)

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · AUGUST 2002

Impact Factor: 2.91 · DOI: 10.1021/jf0203139 · Source: PubMed

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic Profile of Quince Fruit (*Cydonia oblonga* Miller) (Pulp and Peel)

BRANCA M. SILVA,[†] PAULA B. ANDRADE,[†] FEDERICO FERRERES,[‡] ANA L. DOMINGUES,[†] ROSA M. SEABRA,*[,†] AND MARGARIDA A. FERREIRA[§]

Serviço de Farmacognosia and Serviço de Bromatologia, Faculdade de Farmácia, Chemistry Center of Oporto University (CEQUP), Universidade do Porto, R. Aníbal Cunha, 164, 4050-047 Porto, Portugal, and Laboratorio de Fitoquímica, Departamento de Ciencia y Tecnología de los Alimentos, CEBAS (CSIC), P.O. Box 4195, E-30080 Murcia, Spain

Qualitative and quantitative analyses of phenolic compounds were carried out on quince fruit samples from seven different geographical origins in Portugal. For each origin, both pulp and peel were analyzed by reversed-phase HPLC-DAD and HPLC-DAD/MS.The results revealed differences between the phenolic profiles of pulps and peels in all studied cases. The pulps contained mainly caffeoylquinic acids (3-, 4-, and 5-*O*-caffeoylquinic acids and 3,5-dicaffeoylquinic acid) and one quercetin glycoside, rutin (in low amount). The peels presented the same caffeoylquinic acids and several flavonol glycosides: quercetin 3-galactoside, kaempferol 3-glucoside, kaempferol 3-rutinoside, and several unidentified compounds (probably kaempferol glycoside and quercetin and kaempferol glycosides acylated with *p*-coumaric acid). The highest content of phenolics was found in peels.

KEYWORDS: ***Cydonia oblonga* Miller; quince fruit; HPLC-DAD; HPLC-DAD/MS; phenolics**

INTRODUCTION

Quince fruit (*Cydonia oblonga* Miller) is too acid, astringent, and tough to be consumed fresh. However, it can be consumed when cooked or processed as jam or jelly, two food products with great tradition in Portugal. Quince is a seasonal fruit and its homemade jam and jelly are prepared during September and October. According to the Portuguese Legislation, quince jam is the food product of the homogeneous and consistent mixture, obtained exclusively by boiling quince mesocarp with sugars, and quince jelly is the product obtained by cooking a mixture of juice and/or aqueous extract of quince fruit and sugars, in appropriate amounts, with sufficient jellied consistency (*1*).

Phenolic compounds constitute a large and heterogeneous class of compounds with a very wide distribution in taxa of higher plants. Despite this almost ubiquity, experimental evidence has demonstrated that each plant species is characterized by the presence of a limited number of compounds. Within each species, the nature of these compounds can vary from organ to organ but is constant enough toward several other factors. These facts have been used, in recent years, in the characterization of several food products of plant origin by their phenolic profile. Factors contributing to the variability in phenolic distribution include cultivar and genetics, geographical origin, maturity, climate, position on tree, and agricultural practices (*2*).

For quince fruit and its derivatives, few studies have been developed. The usefulness of phenolic compounds in the determination of genuineness of quince puree (*3*), jam (*4, 5*), and jelly (*6*) has been reported. Recently, an HPLC/UV method was developed for the determination of organic acids in quince fruit and its jam (*7*). As far as we know, there are few studies about phenolic composition of quince fruit. Glucosides of procyanidin polymers have been previously identified in this fruit (*8, 9*). The work herein represents a contribution for the chemical characterization of pulp and peel from this fruit. With this purpose, samples of seven different geographical origins from Portugal were analyzed.

MATERIALS AND METHODS

Samples. Healthy quince fruit samples were collected in different places in Northern (Amarante, Baião, Vila Real, and Bragança) and Central Portugal (Viseu, Pinhel and Covilhã), in the year 2000. All fruits were separated into pulp and peel. Each part of the fruit was cut in thin slices and freeze-dried. Lyophilizations were carried out using a Labconco 4.5 apparatus (Kansas City, MO).

Standards. The standards were from Sigma (St. Louis, MO) and Extrasynthése (Genay, France). 3- and 4-*O*-Caffeoylquinic acids were not commercially available, so they were prepared by transesterification of 5-*O*-caffeoylquinic acid (chlorogenic acid) using tetramethylammonium hydroxide (*10, 11*). Methanol, and hydrochloric and formic 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 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).

* To whom correspondence should be addressed. Phone: 351 22 207 8934. Fax: 351 22 200 3977. E-mail: rseabra@ff.up.pt.
[†] Serviço de Farmacognosia, CEQUP.
[‡] CEBAS (CSIC).
[§] Serviço de Bromatologia. CEQUP.

10.1021/jf0203139 CCC: $22.00
Published on Web 06/26/2002

Extraction of Phenolic Compounds. The extraction of phenolics was achieved as previously reported (*5*): each sample (ca. 1 g) was thoroughly mixed with water (pH 2 with HCl) until complete extraction of the phenolic compounds (negative reaction to NaOH 20%) and filtered. 1% Methanol was added to the filtrate, which was then passed through an ISOLUTE C18 (NEC) column which had been preconditioned with 60 mL of methanol and 140 mL of water (pH 2 with HCl). Sugars and other polar compounds were eluted with the aqueous solvent. The retained phenolic fraction was then eluted with methanol (ca. 50 mL). The extract was concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (1 mL).

HPLC-DAD/MS System for Qualitative Analysis. Chromatographic separation was carried out on a LiChroCART column (250 × 4 mm, RP-18, 5-μm particle size, Merck, Darmstadt, Germany) using two solvents: 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. The flow rate was 0.9 mL/min, and the injection volume was 80 μL. Detection was carried out at 270, 320, and 350 nm.

The HPLC system was equipped with a DAD and mass detector in series (Agilent 1100 Series LC/MSD Trap). It consisted of an Agilent G1312A HPLC binary pump, an Agilent G1313 A autosampler, an Agilent G1322A degasser, and an Agilent G1315B photodiode array detector controlled by Agilent software v. A.08.03 (Agilent Technologies, Waldbronn, Germany). The mass detector was an Agilent G2445A ion-trap mass spectrometer equipped with an electrospray ionization (ESI) system and controlled by Agilent Software v. 4.0.25. Nitrogen was used as nebulizing gas at a pressure of 65 psi and the flow was adjusted at 11 L/min. The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. The full scan mass spectra of the phenolic compounds were measured from *m*/*z* 100 up to *m*/*z* 2000. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 100%. Mass spectrometry data were acquired in the negative ionization mode.

HPLC-DAD System for Quantitative Analysis. Separation of the phenolics was achieved as reported previously (*3*−*6*), with an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 × 0.46 cm; 5-μm particle size) column. Detection was achieved with a Gilson 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 Unipointsystem software (Gilson Medical Electronics, Villiers le Bel, France).

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. The other compounds were quantified as themselves.

RESULTS AND DISCUSSION

Qualitative Analysis. Phenolic compounds were identified by comparison of their retention times, UV−Vis spectra in the 200−400 nm range, and MS with those obtained from standards. The identity of 3,5-dicaffeoylquinic acid was confirmed by comparison of its UV spectra and MS and chromatographic behavior with those obtained from the same compound identified in *Cynara cardunculus* (*12*).

All quince pulps presented the same chemical profile, composed by at least five identified phenolic compounds: 3-, 4-, and 5-*O*-caffeoylquinic acids, 3,5-dicaffeoylquinic acid, and rutin (**Figure 1**). The compounds previously identified in pulps were also present in peels. In this last matrix, the DAD signal data recorded at 350 nm can differentiate two groups of peaks between 39 and 62 min. The first group (39−46 min) was formed by five major peaks (peaks 5 to 9) and the second group (51−56 min) was formed by three peaks (**Figure 2**). All of the

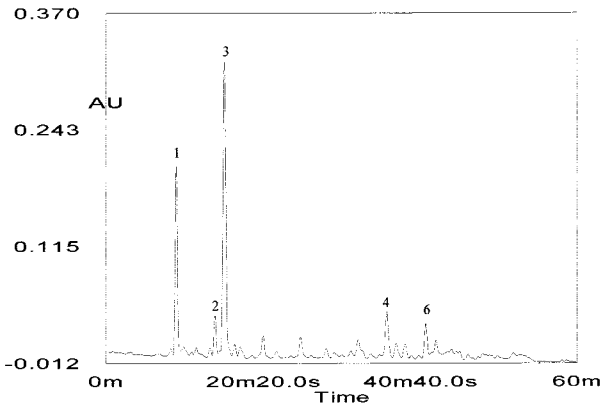

Figure 1. HPLC phenolic profile of a quince pulp. Detection at 350 nm. Peaks: (1) 3-*O*-caffeoylquinic acid; (2) 4-*O*-caffeoylquinic acid; (3) 5-*O*-caffeoylquinic acids; (4) 3,5-dicaffeoylquinic acid, and (6) rutin.

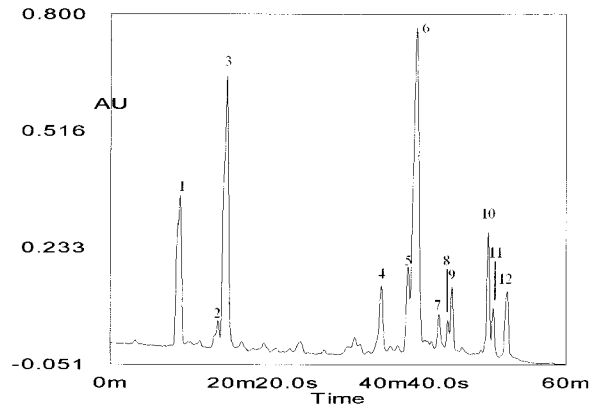

Figure 2. HPLC phenolic profile of a quince peel. Detection at 350 nm. Peaks: (1) 3-*O*-caffeoylquinic acid; (2) 4-*O*-caffeoylquinic acid; (3) 5-*O*-caffeoylquinic acids; (4) 3,5-dicaffeoylquinic acid; (5) quercetin 3-galactoside; (6) rutin; (7) kaempferol glycoside; (8) kaempferol 3-glucoside; (9) kaempferol 3-rutinoside; (10) and (11) quercetin glycosides acylated with *p*-coumaric acid, and (12) kaempferol glycoside acylated with *p*-coumaric acid.

peaks showed identical spectral profile, with two maxima at 257 and 353−355 nm, which indicated that they could be flavonols or flavonol derivatives. HPLC-MS analyses provided interesting information on the two mentioned groups of flavonoids. Pseudomolecular ions $[M - H]^-$ at *m*/*z* 463.8 and 609.5 were found for the peaks 5 and 6, respectively (**Figure 2**). Fragmentation of these ions provided a characteristic *m*/*z* at 300.9, typical mass in the negative mode of the quercetin aglycon. Injection of authentic standards of quercetin 3-galactoside and quercetin 3-rutinoside confirmed the occurrence of these compounds in the quince peel extracts. These results were in agreement with those of previous studies, which described these flavonols in quince puree (*3*), jam (*4*, *5*), and jelly (*6*). Peak 8 (**Figure 2**) had a pseudomolecular ion $[M - H]^-$ at *m*/*z* 448. MS of 448 event yielded main ion at *m*/*z* at 284.6, characteristic of kaempferol, which suggests the presence of a hexosyl kaempferol. The injection of authentic standard of kaempferol 3-glucoside confirmed the presence of this compound in the peels. Peaks 7 and 9 (**Figure 2**) had the same $[M - H]^-$ at *m*/*z* 593.8, and the event yielded a main ion at *m*/*z* 285.6 characteristic of kaempferol and minor fragments at *m*/*z* 448 corresponding to the loss of rhamnose (*m*/*z* 146) from the molecular ion. Authentic kaempferol 3-*O*-rutinoside was injected alone and with the peel quince extract. Retention time, and UV and MS profiles were the same as those for peak 9 (**Figure 2**).

Table 1. Recoveries of 5-*O*-Caffeoylquinic Acid, Rutin, and Kaempferol 3-Rutinoside from Spiked Pulp and Peel from Quince Fruit (quantification by external standard technique)[a]

fruit part	phenolic compound	present (mg/kg)	added (mg/kg)	found[b] (mg/kg)	SD (mg/kg)	CV (%)	recovery (%)
pulp	5-CQA	79.6	233.2	284.8	0.99	0.35	91.0
	Q-3-rut	5.5	64.8	63.6	0.18	0.28	90.5
	K-3-rut	- - -	- - -	- - -	- - -	- - -	- - -
peel	5-CQA	291.6	324.9	558.6	8.44	1.51	90.6
	Q-3-rut	432.7	216.6	599.3	2.83	0.47	92.3
	K-3-rut	36.9	46.9	84.9	0.66	0.78	101.3

[a] SD, standard deviation; CV, coefficient of variation; 5-CQA, 5-*O*-caffeoylquinic acid; Q-3-rut, rutin; K-3-rut, kaempferol 3-rutinoside. [b] mean value found for three assays for each studied concentration;

Table 2. Phenolic Composition of Pulps and Peels from Quince Fruit[a]

		phenolic compounds (mg/kg)								
fruit part	geographic origin	3-CQA (9 m, 9 s)	4-CQA (14 m, 12 s)	5-CQA (15 m, 22 s)	3,5-diCQA (36 m, 12 s)	Q-3-gal (39 m, 43 s)	Q-3-rut (41 m, 5 s)	K-3-glu (45 m, 2 s)	K-3-rut (45 m, 37 s)	Σ
pulp	Amarante	32.4 (0.46)	6.0 (0.06)	79.6 (1.02)	10.8 (0.08)	nd	5.5 (0.20)	nd	nd	134.3
	Baião	23.9 (0.62)	4.1 (0.37)	98.3 (0.98)	8.5 (0.39)	nd	7.4 (0.83)	nd	nd	142.2
	Vila Real	22.7 (0.32)	4.9 (0.26)	54.7 (0.39)	4.3 (0.11)	nd	1.7 (0.10)	nd	nd	88.3
	Bragança	0.9 (0.01)	0.6 (0.01)	5.6 (0.09)	tr	nd	4.6 (0.08)	nd	nd	11.7
	Covilhã	34.7 (1.10)	5.3 (0.01)	108.0 (0.68)	3.7 (0.10)	nd	4.2 (0.15)	nd	nd	155.9
	Viseu	30.2 (0.02)	5.0 (0.07)	65.7 (1.04)	3.6 (0.17)	nd	5.2 (0.57)	nd	nd	109.7
	Pinhel	56.3 (0.70)	7.3 (0.05)	185.6 (2.31)	6.3 (0.20)	4.2 (0.32)	8.6 (0.06)	nd	nd	268.3
	min	0.9	0.6	5.6	tr	nd	1.7	-	-	11.7
	max	56.3	7.3	185.6	10.8	4.2	8.6	-	-	268.3
	mean	28.7	4.7	85.4	5.3	0.6	5.3	-	-	130.1
	SD	16.56	2.09	55.45	3.56	1.59	2.24	-	-	77.58
peel	Amarante	117.2 (2.82)	16.7 (0.72)	291.6 (0.91)	38.3 (1.74)	26.1 (0.60)	432.7 (47.62)	16.7 (0.60)	36.9 (0.61)	976.2
	Baião	32.8 (1.34)	5.7 (0.16)	180.3 (10.10)	16.0 (0.40)	252.0 (8.48)	872.5 (20.35)	92.9 (2.64)	140.2 (1.85)	1592.4
	Vila Real	69.0 (1.72)	10.9 (0.15)	153.4 (4.69)	15.4 (0.73)	48.3 (1.66)	223.2 (9.17)	5.2 (0.14)	13.1 (0.51)	538.5
	Bragança	0.3 (0.02)	0.3 (0.01)	5.9 (0.04)	2.1 (0.13)	33.9 (2.62)	172.3 (1.96)	8.5 (0.11)	20.2 (0.59)	243.5
	Covilhã	11.3 (0.19)	2.4 (0.01)	58.3 (0.94)	5.7 (0.81)	138.1 (1.36)	469.5 (7.28)	45.6 (0.70)	61.2 (0.52)	792.1
	Viseu	56.6 (0.86)	8.8 (0.30)	134.5 (1.42)	13.7 (0.63)	tr	614.7 (10.76)	37.2 (0.46)	79.7 (1.14)	945.2
	Pinhel	101.5 (3.30)	20.4 (0.81)	432.6 (17.25)	22.8 (0.64)	207.2 (6.10)	836.0 (24.18)	41.8 (0.71)	76.3 (1.29)	1738.6
	min	0.3	0.3	5.9	2.1	tr	172.3	5.2	13.1	243.5
	max	117.2	20.4	432.6	38.3	252.0	872.5	92.9	140.2	1738.6
	mean	55.5	9.3	179.5	16.3	100.8	517.3	35.4	61.1	975.2
	SD	44.01	7.33	143.85	11.89	98.78	274.53	30.11	43.58	536.07

[a] Values are expressed as mean (standard deviation) of three assays for each sample. Abbreviations: nd, not detected; tr, traces; 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-dicaffeoylquinic acid; Q-3-gal, quercetin 3-galactoside; Q-3-rut, rutin; K-3-glu, kaempferol 3-glucoside; K-3-rut, kaempferol 3-rutinoside. Retention times are given in parentheses below each phenolic compound abbreviaiton.

Therefore, peak 9 was identified as kaempferol 3-*O*-rutinoside, whereas peak 7 is probably an isomer of compound 9.

Peaks 10−12 (**Figure 2**) had UV spectra typical of acylated flavonoids with a cinnamic acid type (10 and 11, UV 256 shoulder, 266, 294 shoulder, 314, 358 shoulder nm; 12, UV 266, 296 shoulder, 314, 256 shoulder nm). Their $[M - H]^-$ (m/z = 609.8 for compounds 10 and 11 and m/z = 593.8 for compound 12) correspond to a quercetin + hexose + *p*-coumaric acid and a kaempferol + hexose + *p*-coumaric acid, respectively.

A quince peel extract was submitted to a saponification with NaOH 4 N, for 8 h in the presence of N_2, in the dark, at room temperature. The product was directly injected, after filtration through 0.45 μm, into the HPLC-DAD/MS for cinnamic acid and flavonoid glycosides analysis. As a result of this saponification we obtained caffeic acid (from caffeoylquinic acids) (m/z 135, M-45) and *p*-coumaric acid (probably from the previously referenced acylated flavonoids, peaks 10−12) (m/z 119, M-45), hexosyl quercetin, rutin, hexosyl kaempferol, and rhamnohexosyl kaempferol. Meanwhile, peaks 10−12 disappeared from the chromatogram after saponification, which means that they are probably quercetin and kaempferol glycosides acylated with *p*-coumaric acid.

So, quince peels were characterized by the presence of eight identified phenolics: 3-, 4-, and 5-*O*-caffeoylquinic acids, 3,5-dicaffeoylquinic acid, quercetin 3-galactoside, rutin, kaempferol 3-glucoside, and kaempferol 3-rutinoside, and several non-fully identified compounds (probably kaempferol glycoside and quercetin and kaempferol glycosides acylated with *p*-coumaric acid) (**Figure 2**).

As pear and apple also belong to the *Rosaceae* family, it is important to compare the phenolic profiles of these three fruits. Apple is characterized by the presence of (+)-catechin, 5-*O*-caffeoylquinic acid, (−)-epicatechin, *p*-coumaric acid, phloretin 2′-xylosylglucoside, phloretin 2′-glucoside, rutin, quercetin 3-xyloside, and quercetin 3-rhamnoside (*2*, 3). Pear contains 3-*O*-caffeoylquinic acid, (+)-catechin, *p*-hydroxybenzoic acid, 5-*O*-caffeoylquinic acid, (−)-epicatechin, quercetin 3-galactoside, quercetin 3-rhamnoside, and arbutin (*2*, 3). These two fruits have some compounds that can be considered their chemical markers: phloretin 2′-xylosylglucoside and phloretin 2′-glucoside for apple, and arbutin for pear (3−6). So, the addition of apple or pear to quince purees, jams, and jellies can be easily detected by the presence of their characteristic compounds.

From the available literature (*4*, *5*) on quince jams, we verified that in most samples the described profile (based on HPLC-

DAD analysis) was composed by 3-, 4-, and 5-*O*-caffeoylquinic acids, quercetin-3-galactoside, rutin, quercetin-3-xyloside, and quercetin-3-rhamnoside. The present work, in which MS was added to the analytical tools, makes us to think that, most probably, the compounds previously identified as quercetin 3-xyloside and quercetin-3-rhamnoside were not correctly identified, and they correspond to peaks 7 (unidentified kaempferol glycoside) and 8 (kaempferol 3-glucoside) from **Figure 2** (phenolic profile of a quince peel). Some of the compounds present in jams are characteristic of quince peel, which suggests that the manufacturers use all of the quince fruit, and not only the pulp as recommended by Portuguese Legislation (*1*).

Quantitative Analysis. Recently an HPLC-DAD method was developed for determination of the phenolic profile in quince jams (*5*). Once we were dealing with similar matrixes, this technique was applied to quince pulps and peels. Given the similarity of the chemical structures between the several caffeoylquinic acids, quercetin, and kaempferol glycosides, and, therefore, their UV spectra and absorptivity, the recoveries of the extractive method were determined only for 5-*O*-caffeoylquinic acid and rutin in quince pulp and peel, and kaempferol 3-rutinoside in peel. So, to test the recovery of the procedure, one quince pulp and one peel were added to known quantities of each one of the reference phenolics. The samples were analyzed in triplicate before and after the additions. Recovery values were high (between 90.5 and 101.3%) (**Table 1**). This procedure demonstrated the effectiveness of the extraction and the accuracy of the method.

In quince pulps, the most abundant phenolic was 5-*O*-caffeoylquinic acid. Generally, except for the pulp from Bragança, the second major compound was 3-*O*-caffeoylquinic acid (**Table 2**).

The most abundant compound in quince peels was either 5-*O*-caffeoylquinic acid or different flavonol derivatives according to geographic origin. In all cases, quince peel had a higher amount of phenolics than was found in quince pulp (**Table 2**).

In conclusion, this study suggests that the analysis of the phenolic compounds in quince fruit is quite useful in the characterization of its pulp and peel, allowing the discrimination of these two parts of the fruit. Quince pulp has a much lower amount of phenolics than quince peel. Besides, quince pulp and peel differ distinctly in their phenolic profiles: the pulp contains mainly caffeoylquinic acids, whereas the peel possesses both caffeoylquinic acids and several flavonol glycosides, in great amounts.

This procedure also allows the detection of adulterations in quince jams by addition of quince peel.

LITERATURE CITED

(1) Decreto-Lei no. 97/94 de 28 de Março. *Diário da República - I Série B* **1984**, *74*, Portugal.

(2) Spanos, G. A.; Wrolstad, R. E. Phenolics of apple, pear, and white grape juices and their changes with processing and storage − a review. *J. Agric. Food Chem.* **1992**, *40*, 1478−1487.

(3) Andrade, P. B.; Carvalho, A. R. F.; Seabra, R. M.; Ferreira, M. A. A previous study of phenolic profiles of quince, pear, and apple purees by HPLC diode array detection for the evaluation of quince puree genuineness. *J. Agric. Food Chem.* **1998**, *46*, 968−972.

(4) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Valentão, P.; Seabra, R. M.; Ferreira, M. A. Analysis of phenolic compounds in the evaluation of commercial quince jam authenticity. *J. Agric. Food Chem.* **2000**, *48*, 2853−2857.

(5) Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A. Determination of selected phenolic compounds in quince jams by solid-phase extraction and HPLC. *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24* (18), 2861−2872.

(6) Silva, B. M.; Andrade, P. B.; Valentão, P.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Phenolic profile in the evaluation of commercial quince jellies authenticity. *Food Chem.* **2000**, *71*, 281−285.

(7) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Study of the organic acids composition of quince (*Cydonia oblonga* Miller) fruit and jam. *J. Agric. Food Chem.* **2002**, *50*, 2313−2317.

(8) Macheix, J.-J.; Fleuriet, A.; Billot, J. The main phenolics of fruit. In *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; p 87.

(9) Porter, L. J.; Foo, L. Y.; Furneaux, R. H. Isolation of three naturally occurring *O*-*β*-glucopyranosides of procyanidin polymers. *Phytochemistry* **1985**, *24*, 567−569.

(10) Clifford, M. N.; Kellard, B.; Birch, G. G. Characterisation of chlorogenic acids by simultaneous isomerisation and transesterification with tetramethylammonium hydroxide. *Food Chem.* **1989**, *33*, 115−123.

(11) Clifford, M. N.; Kellard, B.; Birch, G. G. Characterisation of caffeoylferuoylquinic acids by simultaneous isomerisation and transesterification with tetramethylammonium hydroxide. *Food Chem.* **1989**, *34*, 81−88.

(12) Seabra, R.; Valentão, P.; Ferreres, F.; Andrade, P. Phenolic profiles in the definition of natural products authenticity. In *Natural Products in the New Millennium: Prospects and Industrial Applications* (in press).

Received for review March 13, 2002. Revised manuscript received May 17, 2002. Accepted May 22, 2002. B.M.S. is grateful to Fundação para a Ciência e a Tecnologia for a grant (PRAXIS XXI/BD/21339/99).

JF0203139