Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 100 (2007) 331–338
Food Chemistry
www.elsevier.com/locate/foodchem

Homo-monoterpenic compounds as chemical markers for *Cydonia oblonga* Miller

Carla Sousa [a], Branca M. Silva [a], Paula B. Andrade [a], Patrícia Valentão [a], Artur Silva [b], Federico Ferreres [c], Rosa M. Seabra [a,*], Margarida A. Ferreira [d]

[a] *REQUIMTE, Serviço de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 4050-047 Porto, Portugal*
[b] *Departamento de Química, Universidade de Aveiro, 3810 Aveiro, Portugal*
[c] *Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Science and Technology, CEBAS-CSIC, P.O. Box 164, E-30100 Espinardo (Murcia), Spain*
[d] *REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 4050-047 Porto, Portugal*

Received 7 March 2005; received in revised form 30 September 2005; accepted 30 September 2005

Abstract

In the course of a phytochemical study of *Cydonia oblonga* Miller, a homo-monoterpenic compound (*trans*-9-amino-8-hydroxy-2,7-dimethylnona-2,4-dienoic acid glucopyranosyl ester) was isolated and identified by hydrolytic and spectroscopic means (nuclear magnetic resonance, mass spectroscopy and UV). Two other chemically related compounds were also detected. Subsequently, these compounds were quantified by HPLC/DAD (diode-array detector) in several pulps, peels and jams. These compounds are described for the first time in nature and can be used as a tool for the characterization of quince and its jam.

Keywords: *Cydonia oblonga* Miller; Quince; Homo-monoterpenic glycosides; Chemical markers

1. Introduction

Quince fruits (*Cydonia oblonga* Miller), when ripe, are characterized by an agreeable, long lasting, and powerful flavour (Schreyen, Dirinck, Sandra, & Schamp, 1979). As they are not edible due to their very hard, tough, and fibrous consistency, they are used for preparing jam. The powerful and characteristic odour of quince fruit is due to the presence of an essential oil (Schreyen et al., 1979; Tsuneya, Ishihara, Shiota, & Shiga, 1983) in which the characteristic compounds are thought to be monoterpenic lactones and oxides (Tsuneya, Ishihara, Shiota, & Shiga, 1980; Tsuneya et al., 1983). In the early nineties, Lutz, Winterhalter, and Schreier (1991) Winterhalter, Lutz, and Schreier (1991) described the existence of monoterpenic glucosides in quince, that were considered to be the precursors of the above lactones and oxides.

Additionally, other phytochemical studies were done on quince (Andrade, Carvalho, Seabra, & Ferreira, 1998; Ferreres, Silva, Andrade, Seabra, & Ferreira, 2003; Silva, Andrade, Mendes, Seabra, & Ferreira, 2002b; Silva, Andrade, Seabra, & Ferreira, 2001; Silva et al., 2000, 2002a, 2003, 2004a, 2004b, 2005). Several analytical methods were developed to determine phenolics, organic acids and free amino acids in quince fruit (pulp, peel and seed) and jam, and their composition, in terms of these compounds, was established (Andrade et al., 1998; Ferreres et al., 2003; Silva et al., 2000, 2001, 2002a, 2002b, 2003, 2004a, 2005). The influence of jam processing upon the contents of these compounds in quince fruit was evaluated (Silva et al., 2004b).

In the course of our study concerning the phenolic profile of quince and its jam, a few compounds, noticed on the chromatograms recorded at 280 nm (with identical UV spectra when recorded with a DAD and presenting a maximum at

* Corresponding author. Tel.: + 351 222078934; fax: +351 222003977.
E-mail address: rseabra@ff.up.pt (R.M. Seabra).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.09.055

approximately 270 nm) (Andrade et al., 1998; Silva et al., 2000, 2001, 2002a), remained unidentified. The paper herein discusses the chemical nature of these compounds. The amounts of three of the mentioned compounds, in several samples (quince pulps, peels and jams), were also evaluated by an HPLC/DAD method.

2. Materials and methods

2.1. Samples

Healthy quince fruit samples were collected in different places in Northern (Amarante, Baião, Vila Real, Bragança, Custóias and Caminha) and Central Portugal (Viseu, Pinhel and Covilhã), in the years of 2000, 2001 and 2002. All fruits were separated into pulp and peel. Each part of the fruit was cut into thin slices and freeze-dried. Lyophilizations were carried out using a Labconco 4.5 apparatus (Kansas City, USA).

Twenty quince jam samples, including four homemade (samples A–D) and 16 industrially manufactured (samples E–T) were examined. The commercial samples were randomly purchased on the Portuguese market, in 2000, 2001 and 2002. A quince jam (jam U) was prepared in the laboratory by boiling fresh quince pulp (from Amarante, year 2002) with sugar (in the proportion of 50:50), for approximately 90 min. Another quince jam (jam V) was similarly prepared, but using unpeeled quinces (from Amarante, year 2002).

2.2. Standards

Citral was from Sigma (St. Louis, MO, USA), Amberlite XAD 2 was from Fluka chemicals (Buchs, Switzerland) and methanol, formic and hydrochloric acids were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Isolation procedures

For isolation purposes ca. 110 g of lyophilized quince pulp from Bragança (year 2000) were used. Aliquots of 10 g of the powdered material were extracted twice with 750 ml of methanol, for 60 min, with agitation. The extracts were combined, filtered and the methanol was removed under reduced pressure (40 °C). The dried extract was thoroughly mixed with acidified water (pH 2 with HCl, 250 ml). Aliquots of 25 ml of this aqueous solution were passed through a column (300 × 30 mm) of Amberlite XAD 2 (pore size 9 nm, particle size 0.3–1.2 mm). The column was washed with acidified water (pH 2 with HCl, 200 ml) and subsequently with distilled water to neutral pH (ca. 1000 ml). Sugars and other polar compounds were eluted with the polar solvent. The compounds retained in the column were then eluted with methanol, for complete extraction of phenolic compounds (ca. 500 ml), until negative reaction with 20% (w/v) NaOH. The methanolic extract was concentrated to approximately 75 ml, at 40 °C, under reduced pressure and filtered. Aliquots of 15 ml of the concentrated extract were passed through a Sephadex LH-20 column (300 × 30 mm) (Sigma Chemical Co., St. Louis, MO: particle size 25–100 μm) eluting with methanol, to give four fractions. The first fraction, with a light colour, and the fourth fraction, with a yellowish colour, were discarded after HPLC analysis (the first fraction contained mainly the already identified flavonoids and the fourth fraction contained mainly caffeoylquinic acids). Fractions 2 and 3, with a brownish colour, were further separated in a LOBAR RP18 column (310 × 25 mm; particle size 40–63 μm), using aliquots of 2.5 ml of the sample. The elution system was methanol/water starting with 10% methanol and with an increment of 10% every 10 min, up to 100% of methanol, which was maintained for 60 min. Fractions were collected for 40 min after the elution started, every 5 min, and analyzed by HPLC. The fractions collected between 1 h 45 min and 2 h 10 min (collected each 10 min) were further purified by semi-preparative HPLC, using a reversed-phase Spherisorb ODS2 column (250 × 10 mm; 10 μm, particle size). The solvent system was water (A) and methanol (B), starting with 25% methanol, and installing a gradient to obtain 30% B at 18 min, 35% B at 30 min and 100% B at 34 min. Elution was performed at a solvent flow rate of 3.0 ml/min; the fractions were manually collected following detection at 280 mn. Three compounds were isolated: compound **a** eluted at 15.7 min, compound **b** at 25.9 min and compound **c** at 25.9 min.

2.4. Acid treatment of compounds

One ml of 2 N HCl was added to 250 μl of each isolated compound or methanolic extract. The acid solution was heated at 100 °C for 30 min and passed through an ISOLUTE C18 column (500 mg sorbent mass/6 ml reservoir volume), previously conditioned with 5 ml of methanol and 5 ml of acid water (pH 2 with HCl) and the compounds were eluted with 5 ml of methanol. The methanolic extract was taken to dryness, redissolved in 250 ml of methanol, and by 20 ml were analysed by HPLC.

2.5. Alkaline treatment

This was performed by adding 1 ml of 2 N NaOH to 250 μl of each isolated compound solution or methanolic extract and keeping the mixture for 4 h at room temperature, in the dark. After this step, the alkaline hydrolysis products were acidified with concentrated HCl (up to pH 1–2) and passed through an ISOLUTE C18 column (500 mg sorbent mass/6 ml reservoir volume), previously conditioned with 5 ml of methanol and 5 ml of acid water (pH 2 with HCl). The compounds were eluted with 5 ml of methanol. The methanolic extract was taken to dryness, redissolved in 250 ml of methanol, and by 20 ml were analysed by HPLC.

2.6. Spectrometric analysis

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Brucker Avance-500 instrument, operating at 500 and 125 MHz, respectively, and tetramethylsilane (TMS) was used as an internal standard. The MS spectrum was recorded in an AutoSpecEQ apparatus.

2.7. *trans*-9-Amino-8-hydroxi-2,7-dimethylnona-2,4-dienoic acid glucopyranosyl ester (**a**)

UV (MeOH): $\lambda_{max} = 274$ nm. FAB-MS (positive ion): m/z 376 $[M + H]^{+}$.

^{1}H NMR (DMSO-d_6): δ 0.80 (3H, d, $J = 6.6$ Hz, CH_3-C7), 1.57 (1H, m, H-C7), 1.87 (3H, s, CH_3-C2), 1.99 (1H, m, H-C6), 2.11 (1H, m, H-C9), 2.31 (1H, m, H-C9), 2.38 (1H, m, H-C6), 3.12 (1H, m, H-C4′), 3.18 (1H, m, H-C2′), 3.20 (1H, m, H-C5′), 3.23 (1H, m, H-C3′), 3.44 (1H, m, H-C6′), 3.62 (1H, m, H-C8), 3.64 (1H, m, H-C6′), 5.38 (1H, d, $J = 7.9$ Hz, H-C1′), 6.21 (1H, m, H-C5), 6.45 (1H, t, $J = 14.5$, 11.7 Hz, H-C4), 7.21 (1H, d, $J = 11.3$ Hz, H-C3).

^{13}C NMR (DMSO-d_6): δ 12.4 (CH_3-2), 15.4 (CH_3-7), 35.8 (C-6), 38.6 (C-7), 40.18 (C-9), 60.5 (C-6′), 69.4 (C-4′), 70.9 (C-8), 72.5 (C-2′), 76.4 (C-3′)[a], 77.8 (C-5′)[a], 94.5 (C-1′), 123.6 (C-2), 127.0 (C-4), 139.8 (C-3), 143.7 (C-5), 166.4 (C-1). [a]Assignments are interchangeable.

2.8. Extraction for quantification purposes

The extraction was achieved as previously reported (Silva et al., 2001, 2002a, 2004b, 2005) and included a C18 SPE cleaning step.

2.9. HPLC analysis

The extracts (20 μl) were analyzed as previously described (Andrade et al., 1998; Silva et al., 2000, 2001, 2002a, 2004b, 2005), on an analytical HPLC unit (Gilson), using an Spherisorb ODS2 column (25.0 × 0.46 cm; 5 μm, particle size). Detection was achieved with a Gilson DAD.

2.10. Quantification

In each extract, compounds **a**, **b** and **c** were localized by comparing their retention times and UV–Vis spectra, with those of the now isolated compounds. Peak purity was checked by means of the Gilson 160 spectra viewer software contrast facilities. Quantification of these compounds

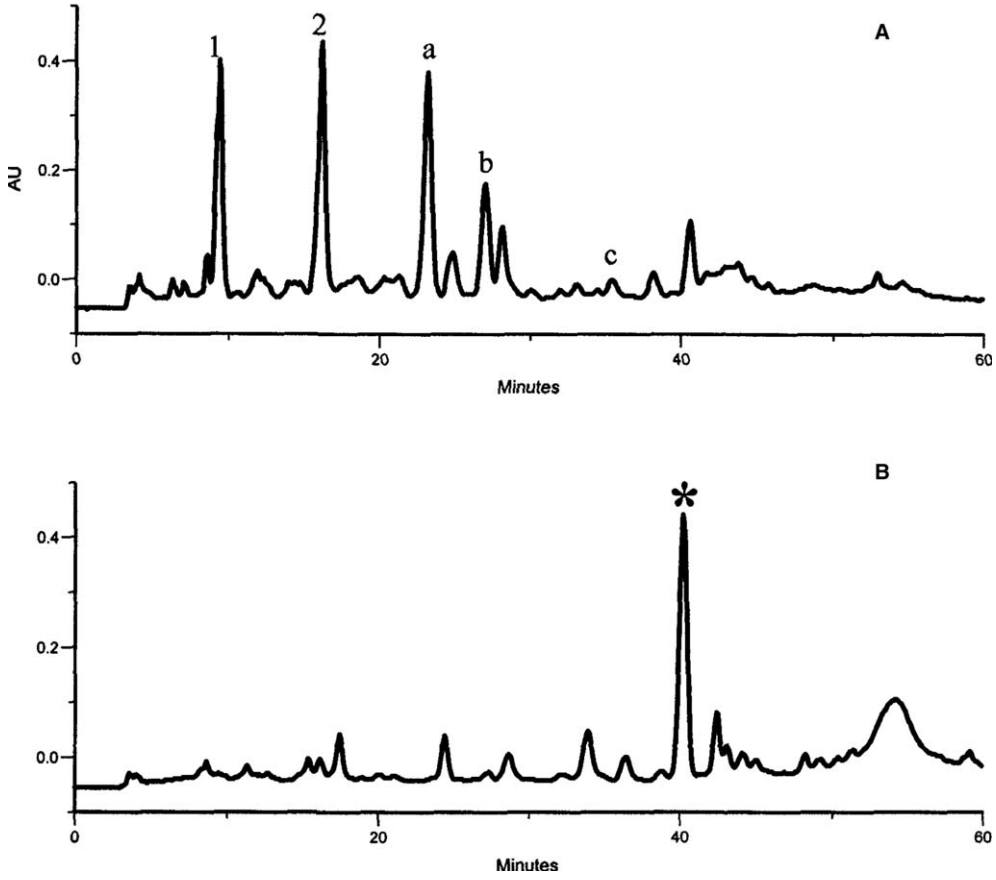

Fig. 1. HPLC profile of the methanolic extract of quince pulp from Pinhel (2002) (A) and the result of the alkaline hydrolysis of the same extract (B). Detection at 280 nm. Peaks: (**1**) 3-*O*-caffeoylquinic acid, (**2**) 5-*O*-caffeoylquinic acid, (**a**), (**b**) and (**c**) isolated compounds and ∗ resulting compound from alkaline hydrolysis.

was achieved by the absorbance recorded in the chromatograms relative to external standard, with detection at 280 nm. All of the compounds were quantified as citral.

3. Results and discussion

3.1. Characterization of isolated compounds

In previous studies concerning quince phenolics (Andrade et al., 1998; Silva et al., 2000, 2001, 2002a), some compounds, detected in the chromatograms recorded at 280 nm, remained unidentified (Fig. 1A). They all showed UV spectra with only one maximum, around 270 nm, and, according to some published data, they were assigned as possible procyanidin polymers (Andrade et al., 1998). A methanolic extract of quince pulp was now subjected to several procedures in order to isolate them and three compounds could be obtained in adequate purity to attempt the identification.

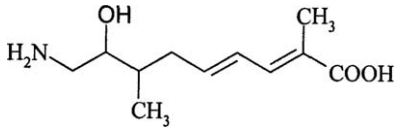

Fig. 2. Chemical structure of the aglycone of compounds **a, b** and **c**, isolated from *Cydonia oblonga* Miller.

The UV spectra of compound **a** exhibited only one maximum at 274 nm. The results obtained from the ^{1}H NMR spectrum indicated the presence of one methyl group in the aliphatic region (δ 0.80, 3H, d, $J = 6.6$ Hz), another methyl group attached to an unsaturated carbon (δ 1.87, 3H, s), a conjugated alkene (δ 6.21, 1H, m; 6.45, 1H, dd, $J = 14.5$, 11.7 Hz; 7.21, 1H, d, $J = 11.3$ Hz), four methylene protons (δ 1.99, 1H, m; 2.11, 1H, m; 2.31, 1H, m; 2.38, 1H, m) and a methine proton (δ 1.57, 1H, m). Another methine proton was detected at δ 5.39 ($J =$ 7.9 Hz) and a series of signals between δ3.12 and 3.64, indicating the presence of a β-glycosidic moiety in the molecule. In the ^{13}C NMR spectra, signals were detected for one carbonylic group (δ 166.4), two methyl groups (δ 12.4 and 15.4), four carbon atoms involved in a conjugated alkene structure (δ 123.6; 127.0; 139.8; 143.7), one methine carbon (δ 38.6) and two methylene carbons (δ 35.8 and 70.9). The high value found for the carbon atom of the methylene group, at δ 70.9, indicated the existence of an

Table 1
Isolated homo-monoterpenes composition of quince pulps

Geographic origin	Year	Isolated compounds (mg/kg)						∑
		Compound **a**[a]	SD	Compound **b**[a]	SD	Compound **c**[a]	SD	
Amarante	2000	99.1	0.09	41.3	0.16	5.94	0.13	146
	2001	44.7	0.58	6.99	0.10	8.96	0.08	60.6
	2002	33.5	0.63	23.1	0.60	2.05	0.03	58.7
Baião	2000	65.4	0.43	31.4	0.68	5.05	0.15	102
	2001	60.0	0.86	15.2	0.04	10.2	0.01	85.3
	2002	51.5	2.00	38.7	0.52	20.1	0.12	110
Vila Real	2000	50.7	0.58	16.9	0.04	2.27	0.01	69.8
	2001	74.5	1.12	30.9	0.18	10.5	0.03	116
	2002	84.5	1.41	31.3	0.00	23.5	0.32	139
Bragança	2000	45.2	0.68	37.5	0.97	16.1	0.52	98.7
	2001	53.1	0.15	20.6	0.19	11.3	0.03	84.9
	2002	24.4	0.44	21.8	0.37	16.3	0.47	62.4
Covilhã	2000	48.3	0.22	24.5	1.45	5.36	0.09	78.2
	2001	101	0.45	36.3	1.96	14.1	0.05	151
	2002	37.3	1.96	16.8	0.48	36.7	0.81	90.8
Viseu	2000	59.8	1.06	29.5	0.38	5.89	0.05	95.2
	2001	90.8	2.20	49.4	1.41	21.9	0.12	162
	2002	59.2	0.56	28.4	0.53	28.6	0.32	116
Pinhel	2000	80.9	0.01	48.5	0.73	4.14	0.36	134
	2001	59.9	0.23	47.2	0.34	19.0	0.18	126
	2002	81.1	2.27	43.6	1.77	28.4	0.45	153
Custóias	2001	3.69	0.06	17.2	0.05	14.6	1.09	35.5
	2002	54.7	0.21	12.5	0.17	20.0	0.52	87.2
Caminha	2001	52.2	0.95	23.4	0.82	9.79	0.20	85.4
Mean		59.0		28.9		14.2		102
Maximum		101		49.4		36.7		162
Minimum		3.69		6.99		2.05		35.5
SD		23.2		12.0		9.17		33.9

[a] Values are expressed as means of three determinations; SD, standard deviation; ∑, sum of the determined compounds.

oxygenated group attached to it. Other signals were found which corresponded to the carbons of a glycosidic structure (δ 60.5; 69.4; 72.5; 76.4; 77.8; 94.5), most probably glucose (Markham, Chari, & Mabry, 1982; Markham & Geiger, 1994).

The compound was also subjected to HSQC (heteronuclear single quantum correlation) analysis, which allows unequivocal correlations between each carbon and the protons attached to it. This analysis showed that the methylene protons at δ 2.11 and 2.31 were linked to a carbon, whose chemical shift was overlapped by the signal of DMSO (δ around 40). A new ^{13}C NMR was obtained in $CDCl_3$ which guaranteed the existence of such carbon and indicated that compound **a** was a glycosidic ester of an aglycone containing 11 carbon atoms.

The exact sequence of all portions of the molecule was confirmed by subjecting the compound to analysis by COSY (correlated spectroscopy) and HMBC (heteronuclear multiple bond correlation).

The existence of nitrogen in the molecule was deduced from the mass spectrum, which showed the molecular ion at m/z 376. The position of nitrogen at carbon 9 was demonstrated by HMBC and HSQC and by the high chemical shift value of this terminal carbon.

In order to get a deep insight of the behaviour of the compound now isolated, it was subjected to acid treatment. Upon this treatment, compound **a** formed another compound with a higher retention time (40 min) (Fig. 1B), but the UV spectrum remained unchanged. This new compound was subjected to ^1H and ^{13}C NMR ($CDCl_3$). All the data obtained, including those from COSY, HSQC and HMBC, showed that acid hydrolysis of compound **a** formed the compound represented in Fig. 2.

Given the behaviour of compound **a** on alkaline hydrolysis, that is, the break of the ester linkage, and the up-field shift observed on the resonance of carbon 1 after hydrolysis (data not shown), glucose is linked to the aglycone through the carboxyl group. From all these data, compound **a** was identified as *trans*-9-amino-8-hydroxy-2,7-dimethylnona-2,4-dienoic acid glucopyranosyl ester.

HPLC-DAD analysis of compounds **b** and **c** showed only one maximum at 270 and 268, respectively, but they were isolated in too small amounts to try any analysis by NMR. When subjected to acid or alkaline treatment, all

Table 2
Isolated homo-monoterpenes composition of quince peels

Geographic origin	Year	Isolated compounds (mg/kg)						$\sum$
		Compound **a**[a]	SD	Compound **b**[a]	SD	Compound **c**[a]	SD	
Amarante	2000	106	4.17	52.4	2.76	23.5	0.71	182
	2001	56.2	0.14	22.8	0.44	17.4	0.14	96.5
	2002	32.4	0.65	23.9	0.65	9.04	0.43	65.4
Baião	2000	51.1	0.22	43.1	0.18	15.8	0.03	110
	2001	89.0	0.91	28.8	0.16	31.1	0.61	149
	2002	65.3	0.60	56.8	0.52	10.6	0.08	133
Vila Real	2000	61.0	1.01	20.3	0.34	12.7	0.02	94.0
	2001	61.2	0.36	22.3	0.04	33.3	1.12	117
	2002	56.0	0.90	25.2	0.23	16.9	1.33	98.1
Bragança	2000	35.3	1.79	42.9	1.42	8.71	0.14	86.9
	2001	36.4	0.40	9.04	0.64	25.9	1.14	71.3
	2002	26.1	0.49	24.4	0.49	17.1	0.74	67.5
Covilhã	2000	37.1	0.81	38.0	0.47	20.1	0.08	95.1
	2001	55.2	2.50	18.5	0.55	17.8	0.30	91.4
	2002	31.4	0.35	19.6	1.12	74.7	1.73	126
Viseu	2000	67.4	1.41	44.5	1.78	28.9	0.34	141
	2001	47.2	0.25	27.6	1.58	51.7	3.91	127
	2002	52.7	0.18	31.6	0.01	20.8	0.08	105
Pinhel	2000	68.3	1.41	50.9	1.30	20.3	0.21	140
	2001	52.4	2.40	25.9	1.98	30.7	0.73	109
	2002	44.3	0.01	28.3	0.22	14.1	0.78	86.7
Custóias	2001	111.3	1.75	55.0	0.54	51.1	0.08	218
	2002	55.0	1.53	16.7	0.54	6.14	0.26	77.8
Caminha	2001	130	0.50	45.9	0.15	38.9	0.29	215
Mean		59.5		32.3		24.9		117
Maximum		130		56.8		74.7		218
Minimum		26.1		9.04		6.14		65.4
SD		26.1		13.5		16.1		41.5

[a] Values are expressed as means of three determinations; SD, standard deviation; $\sum$, sum of the determined compounds.

Table 3
Isolated homo-monoterpenes composition of quince jams

Jam	Year	Isolated compounds (mg/kg)						$\sum$
		Compound **a**[a]	SD	Compound **b**[a]	SD	Compound **c**[a]	SD	
A	2000	17.2	0.25	12.6	0.18	26.5	1.08	56.2
	2001	18.9	0.32	11.1	0.57	3.10	0.08	33.1
	2002	12.8	0.54	12.8	0.26	7.20	0.17	32.9
B	2000	25.8	0.18	24.6	0.81	17.0	0.81	67.4
	2001	30.6	0.04	11.3	0.25	4.66	0.00	46.6
C	2000	9.30	0.14	3.90	0.10	37.5	0.35	50.7
	2001	22.7	0.43	9.72	0.22	4.50	0.16	36.9
D	2000	38.9	0.63	18.9	0.42	17.2	0.20	75.0
E	2000	29.5	1.24	18.5	0.14	5.43	0.18	53.4
	2001	7.05	0.12	6.25	0.04	7.91	0.22	21.2
	2002	28.5	0.89	15.0	0.21	3.25	0.33	46.8
F	2000	26.0	0.30	47.2	2.59	2.90	0.04	76.0
	2001	18.6	0.27	14.0	0.04	2.74	0.02	35.3
	2002	16.0	0.36	12.6	0.12	2.77	0.05	31.4
G	2000	22.4	0.70	83.3	0.34	3.86	0.10	110
	2001	14.1	0.03	10.1	0.03	7.30	0.01	31.5
	2002	18.8	1.36	7.78	0.11	20.7	0.83	47.3
H	2000	20.3	1.63	25.0	1.67	2.66	0.08	47.9
	2001	6.04	0.10	5.75	0.09	33.6	0.18	45.4
I	2000	20.7	0.60	12.9	0.38	3.10	0.13	36.8
	2001	17.8	0.43	14.6	0.08	17.5	0.41	49.9
	2002	30.3	2.65	21.6	0.61	3.27	0.34	55.1
J	2000	32.4	0.40	22.1	0.46	3.16	0.11	57.7
	2001	13.1	0.02	18.1	0.51	5.21	0.18	36.5
	2002	8.55	0.14	8.88	0.11	8.84	0.12	26.8
K	2000	33.7	0.28	32.0	0.18	3.85	0.23	69.6
	2001	24.8	0.12	14.3	0.01	2.44	0.22	41.6
	2002	31.4	2.43	20.5	0.67	1.92	0.02	53.8
L	2000	18.0	1.37	22.6	0.04	1.77	0.00	42.4
M	2000	42.4	1.56	23.6	0.27	5.49	0.04	71.5
	2001	11.1	0.16	14.8	0.46	25.4	0.35	51.2
	2002	12.9	0.03	11.2	0.13	18.6	0.92	42.7
N	2000	11.4	0.80	42.0	2.47	11.1	0.52	64.4
	2001	19.3	0.02	19.7	0.16	21.0	0.69	59.9
	2002	34.4	1.23	25.2	1.76	5.38	0.12	65.0
O	2000	10.8	0.56	28.3	0.53	12.1	0.16	51.3
	2001	16.4	0.56	12.0	0.40	1.50	0.08	29.9
	2002	25.6	0.00	20.4	0.06	15.7	0.34	61.6
P	2000	14.3	0.13	28.7	0.12	1.23	0.04	44.2
	2001	10.2	0.06	8.86	0.24	2.93	0.02	22.0
	2002	15.3	0.07	12.3	0.08	11.4	0.10	39.0
Q	2000	28.1	0.11	36.7	0.37	3.53	0.05	68.3
	2001	24.8	0.28	15.2	0.06	9.83	0.03	49.8
	2002	33.5	0.53	20.4	0.08	5.06	0.09	58.9
R	2000	39.9	0.23	69.1	0.16	34.9	2.36	144
	2001	10.9	0.04	8.19	0.33	7.92	0.22	27.0
	2002	23.9	0.97	15.8	0.26	2.50	0.00	42.2
S	2000	31.9	0.32	42.5	0.42	4.54	0.05	79.0
T	2000	20.2	0.07	73.2	2.30	4.99	0.10	98.3
U	2002	46.0	0.20	18.0	0.20	2.54	0.17	66.6
V	2002	24.9	0.25	16.4	0.18	2.14	0.18	43.5
Mean		22.0		21.6		9.24		52.8

Table 3 (*continued*)

Jam	Year	Isolated compounds (mg/kg)						∑
		Compound **a**[a]	SD	Compound **b**[a]	SD	Compound **c**[a]	SD	
Maximum		46.0		83.3		37.5		144
Minimum		6.04		3.90		1.23		21.2
SD		9.68		16.5		9.26		22.2

[a] Values are expressed as means of three determinations; SD, standard deviation; ∑, sum of the determined compounds.

of them formed the same compound, with a retention time of 40 min, superimposable on that of compound **a** aglycone, indicating that all these three compounds were, most probably, esters of the same aglycone, represented in Fig. 2. This conclusion can also be drawn from inspection of the methanolic extract chromatogram of a quince pulp sample, where compounds **a**, **b** and **c** are pointed out, (Fig. 1A), and from the chromatogram of the same methanolic extract after alkaline hydrolysis (Fig. 1B). On this last chromatogram the almost total disappearance of compounds, **a**, **b** and **c** can be observed and the appearance of only one compound with a retention time of 40 min.

The existence of a β-glucosidic ester of a terpenic compound has already been reported in quince (Winterhalter et al., 1991), but, in contrast to what happened to that ester, the compounds now described do not lactonize upon hydrolysis. Besides, this is the first report of such a compound including nitrogen in its molecule.

3.2. Quantitative analysis

The compounds characterized in this work are easily observed in a HPLC chromatogram of methanolic extract of quince and can be considered as chemical markers because they form large peaks and are not present in pear or apple (Andrade et al., 1998). Bearing in mind that the chemical characterization of a food product is achieved, not only by the presence of a set of compounds, but also by reference to its amounts or, at least, to its proportions, a quantitative analysis was also carried out in several samples of pulps, peels and jams.

All analyzed samples (pulps, peels and jams) exhibited compounds **a**, **b** and **c**, and their amounts are shown in Tables 1–3. In pulps and peels, compound **a** was usually present in higher amounts than the others. The mean contents of compounds **a** and **b** in pulps and peels were similar (59.0 and 59.5 mg/kg, and 28.9 and 32.3 mg/kg, respectively). Compound **c** was present in higher amounts in peels than in pulps (24.9 and 14.2 mg/kg, respectively).

Jams had generally lower contents of these compounds. Nevertheless, jam U, which was prepared with pulp from Amarante (2002), showed higher amounts of compound **a** than did the correspondent pulp.

Acknowledgements

We thank the Department of Chemistry, University of Aveiro for mass spectra and Branca M. Silva is grateful to Fundação para a Ciência e a Tecnologia for a Grant (PRAXIS XXI/BD/21339/99).

References

Andrade, P. B., Carvalho, A. R. F., Seabra, R. M., & Ferreira, M. A. (1998). A previous study of phenolic profiles of quince, pear, and apple purees by HPLC diode array detection for the evaluation of quince puree genuineness. *Journal of Agricultural and Food Chemistry, 46*, 968–972.

Ferreres, F., Silva, B. M., Andrade, P. B., Seabra, R. M., & Ferreira, M. A. (2003). Approach to the study of C-glycosyl flavones by Ion Trap HPLC-PAD-ESI/MS/MS: application to seeds of quince (*Cydonia oblonga*). *Phytochemical Analysis, 14*, 352–359.

Lutz, A., Winterhalter, P., & Schreier, P. (1991). Isolation of a glucosidic precursor of isomeric marmelo oxides from quince fruit. *Tetrahedron Letters, 32*, 5943–5944.

Markham, K. R., Chari, V. M., & Mabry, T. J. (1982). Carbon-13 NMR spectroscopy of flavonoids. In J. B. Harborne & T. J. Mabry (Eds.), *The flavonoids – Advances in research* (pp. 19–134). London: Chapman and Hall.

Markham, K. R., & Geiger, H. (1994). ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In J. B. Harborne (Ed.), *The flavonoids – Advances in research since 1986* (pp. 441–497). London: Chapman and Hall.

Schreyen, L., Dirinck, P., Sandra, P., & Schamp, N. (1979). Flavor analysis of quince. *Journal of Agricultural and Food Chemistry, 27*, 872–876.

Silva, B. M., Andrade, P. B., Ferreres, F., Domingues, A. L., Seabra, R. M., & Ferreira, M. A. (2002a). Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel). *Journal of Agricultural and Food Chemistry, 50*, 4615–4618.

Silva, B. M., Andrade, P. B., Ferreres, F., Seabra, R. M., Oliveira, M. B., & Ferreira, M. A. (2005). Composition of quince (*Cydonia oblonga* Miller) seeds: phenolics, organic acids and free amino acids. *Natural Product Research, 19*, 275–281.

Silva, B. M., Andrade, P. B., Gonçalves, A. C., Seabra, R. M., Oliveira, M. B., & Ferreira, M. A. (2004b). Influence of jam processing upon the contents of phenolics, organic acids and free amino acids in quince fruits (*Cydonia oblonga* Miller). *European Food Research and Technology, 218*, 385–389.

Silva, B. M., Andrade, P. B., Mendes, G. C., Seabra, R. M., & Ferreira, M. A. (2002b). Study of the organic acids composition of quince (*Cydonia oblonga* Miller) fruit and jam. *Journal of Agricultural and Food Chemistry, 50*, 2313–2317.

Silva, B. M., Andrade, P. B., Mendes, G. C., Valentão, P., Seabra, R. M., & Ferreira, M. A. (2000). Analysis of phenolic compounds in the evaluation of commercial quince jam authenticity. *Journal of Agricultural and Food Chemistry, 48*, 2853–2857.

Silva, B. M., Andrade, P. B., Seabra, R. M., & Ferreira, M. A. (2001). Determination of selected phenolic compounds in quince jams by solid-phase extraction and HPLC. *Journal of Liquid Chromatography and Related Technologies, 24*(18), 2861–2872.

Silva, B. M., Casal, S., Andrade, P. B., Seabra, R. M., Oliveira, M. B., & Ferreira, M. A. (2003). Development and evaluation of a GC/FID

method for the analysis of free amino acids in quince fruit and jam. *Analytical Sciences, 19*, 1285–1290.

Silva, B. M., Casal, S., Andrade, P. B., Seabra, R. M., Oliveira, M. B., & Ferreira, M. A. (2004a). Free amino acid composition of quince (*Cydonia oblonga* Miller) fruit (pulp and peel) and jam. *Journal of Agricultural and Food Chemistry, 52*, 1201–1206.

Tsuneya, T., Ishihara, M., Shiota, H., & Shiga, M. (1980). Isolation and identification of novel terpene lactones from quince fruit (*Cydonia oblonga* Mill., marmelo). *Agricultural and Biological Chemistry, 44*, 957–958.

Tsuneya, T., Ishihara, M., Shiota, H., & Shiga, M. (1983). Volatile components of quince fruit (*Cydonia oblonga* Mill.). *Agricultural and Biological Chemistry, 47*, 2495–2502.

Winterhalter, P., Lutz, A., & Schreier, P. (1991). Isolation of a glucosidic precursor of isomeric marmelo lactones from quince fruit. *Tetrahedron Letters, 32*, 3669–3670.