Analysis of Phenolic Compounds in the Evaluation of Commercial Quince Jam Authenticity

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The phenolic compounds present in 17 samples of Portuguese commercial and three homemade quince jams were analyzed by reversed-phase HPLC/DAD, to determine their authenticity. Two different extraction methods were needed for the complete definition of quince jams profiles, one of them including an Amberlite XAD-2 cleaning step. These analyses showed that all the samples presented a similar profile composed of at least eight identified phenolic compounds, several unidentified characteristic procyanidin polymers, and sodium benzoate as preservative of quince jams. Several samples also contained arbutin, suggesting that these quince jam samples were fraudulently adulterated with pear puree.

Keywords: *Quince; jam; phenolics; HPLC; adulteration.*

INTRODUCTION

Quince jam is a product obtained from the pulp of the fruit of *Cydonia oblonga* Miller, var. *maliformis* or *piriformis*. This foodstuff is industrially manufactured or homemade during the September/October months by boiling a mixture of sugar and quince puree until the appropriate consistency is reached (usually 65−72 °Brix). When quince production is scarce, industry manufacturers are tempted to adulterate quince jam by adding apple (*Malus communis* Lamk) and/or pear (*Pirus communis* Lin.) due to their low cost. From a sensory point of view, this falsification is hardly detectable because of the similar texture of these fruits and the strong odor of quince that easily covers apple and/or pear flavors.

Phenolic compounds are widely distributed in nature and have been successfully used for the determination of genuineness of some fruit jams and juices (Lee and Wrolstad, 1988; Burda et al., 1990; Spanos et al., 1990; Spanos and Wrosltad, 1990 and 1992; Simón et al., 1992; Tomás-Lorente et al., 1992; Tomás-Barberán et al., 1993; Vallés et al., 1994). The usefulness of phenolic profiles in the determination of genuineness of quince puree has been already reported by Andrade et al. (1998). Addition of apple and pear to quince puree can be detected by the presence of their characteristic compounds, phloretin 2′-xylosylglucoside and phloretin 2′-glucoside for apple and arbutin for pear.

The work herein represents a contribution for the definition of the quince jam phenolic profile, to know if it could be used for the detection of apple and/or pear present in quince jam, allowing the determination of its authenticity. With this purpose, several homemade and commercially available samples were analyzed by HPLC/DAD.

MATERIALS AND METHODS

Standards and Reagents. The standards were from Sigma (St. Louis, MO) and from Extrasynthése (Genay, France). 3- and 4-*O*-caffeoylquinic acids were not commercially available, so they were prepared by transesterification of 5-*O*-caffeoylquinic acid using tetramethylammonium hydroxide (Clifford et al., 1989a,b). HPLC-grade methanol and formic acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Samples. Quince jams (three blends of each sample) were purchased in supermarkets. Homemade quince jams were provided from different origins.

Sample Preparation. Phenolics Extraction via the Amberlite XAD-2 Step. Each quince jam (ca. 40 g) was thoroughly mixed with five parts of water (pH 2 with HCl) until completely fluid and filtered through cotton wood to remove solid particles. The filtrate was then passed through a column (25 × 2 cm) of Amberlite XAD-2 (Fluka Chemicals: pore size 9 nm, particle size 0.3−1.2 mm), as reported previously (Ferreres et al., 1994). Sugars and other polar compounds were eluted with the aqueous solvent. The column was washed with water (pH 2 with HCl, 100 mL) and subsequently with distilled water (ca. 300 mL). The phenolic fraction remained in the column and was then eluted with methanol (ca. 300 mL). The methanolic extract was evaporated to dryness under reduced pressure (40 °C) and redissolved in methanol (1.5 mL), and 20 μL was analyzed by HPLC.

Phenolics Extraction via the Simplified Technique. Each quince jam (ca. 40 g) was thoroughly mixed with methanol until complete extraction of phenols (negative reaction with NaOH). The extract was then filtered, evaporated to dryness under reduced pressure (40 °C), and redissolved in methanol (10 mL), and 20 μL was analyzed by HPLC.

HPLC Analysis of Phenolic Compounds. Separation of phenolics was achieved as reported previously (Andrade et al., 1998), with an analytical HPLC unit (Gilson), using a Sperisorb ODS2 (25.0 × 0.46 cm; 5 μm, particle size) column. 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 25min, 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. Detection was achieved with a diode array detector, and chromatograms were recorded at 350 and 280 nm.

The compounds in each sample were identified by comparing their retention times and UV−vis spectra in the 200−400 nm

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Table 1. Phenolic Composition of Quince Jams (mg Phenolic Compound/kg Jam) Obtained by Amberlite XAD-2 Extraction[a]

samples[b]	arbutin[c] (RT 4m52s)	gallic acid[c] (RT 6m4.8s)	3-*O*-caffeoylquinic acid (RT 9m55s)	4-*O*-caffeoylquinic acid (RT 15m32s)	5-*O*-caffeoylquinic acid (RT 16m17s)	rutin (RT 42m0.4s)	quercetin 3-galactoside (RT 42m32s)	quercetin 3-xyloside (RT 44m32s)	quercetin 3-rhamnoside (RT 45m49s)
A1	71.6 (15.77)		21.1 (0.17)	5.9 (0.05)	31.0 (0.19)	0.8 (0.02)	4.1 (0.03)	0.2 (0.006)	0.1 (0.02)
A2	10.6 (1.83)	38.6 (2.21)	12.6 (1.07)	7.8 (0.84)	33.0 (2.40)	nq	6.8 (0.59)	0.2 (0.03)	0.04 (0.01)
A3	16.9 (4.26)		6.3 (0.38)	2.3 (0.15)	27.3 (2.15)	1.2 (0.001)	5.7 (0.41)	0.04 (0.008)	0.04 (0.005)
B1	nq		31.4 (2.36)	7.3 (0.34)	34.3 (1.10)	0.8 (0.03)	4.7 (0.21)	0.3 (0.009)	0.09 (5E−4)
B2	22.5 (2.77)	60.5 (0.74)	4.0 (0.16)	4.1 (0.53)	8.0 (0.03)	0.04 (3E−4)	7.6 (0.40)	0.6 (0.03)	0.5 (0.04)
B3	9.2 (3.25)		10.6 (0.62)	3.0 (0.22)	34.1 (2.29)	0.3 (0.03)	3.5 (0.04)	0.2 (0.03)	0.5 (0.02)
C1	159.9 (3.00)		32.9 (0.79)	15.5 (0.50)	75.4 (1.40)	1.4 (0.004)	6.1 (0.01)	0.1 (0.07)	0.2 (0.03)
C2	45.0 (6.67)	27.2 (4.76)	11.2 (6.40)	5.3 (0.09)	42.8 (1.33)	nq	12.4 (0.98)	0.5 (0.04)	0.03 (0.002)
C3	28.5 (6.70)	7.8 (0.49)	0.5 (0.04)	0.2 (0.03)	6.7 (0.46)	0.3 (0.03)	0.9 (0.09)	0.3 (0.06)	0.7 (0.05)
D1			19.6 (15.65)	21.8 (0.55)	99.5 (0.42)	1.9 (1.12)	11.1 (4.24)	0.8 (0.04)	0.4 (0.06)
D2			3.6 (0.29)	3.2 (0.25)	23.2 (1.21)	nq	4.8 (0.22)	0.06 (0.004)	0.03 (0.004)
E1	19.0 (1.67)		34.7 (0.51)	20.2 (0.25)	100.6 (7.35)	3.3 (0.09)	25.9 (0.37)	0.4 (9E−4)	0.2 (0.09)
E2	18.0 (2.69)		5.9 (0.71)	6.2 (0.05)	40.3 (2.54)	nq	16.3 (1.09)	0.7 (0.05)	0.08 (9E−4)
E3	37.7 (3.28)		0.3 (0.01)	nq	6.1 (0.63)	0.09 (5E−4)	0.2 (0.005)	0.09 (0.002)	0.4 (0.02)
F1	59.3 (2.18)		4.4 (0.16)	2.2 (0.07)	17.5 (0.67)	0.1 (0.01)	0.9 (0.03)	0.1 (0.01)	0.03 (0.002)
F2	21.0 (2.24)		2.7 (0.11)	1.3 (0.08)	11.6 (0.19)	0.07 (0.001)	3.6 (0.05)	0.1 (0.02)	0.04 (4E−4)
F3	18.6 (0.09)		10.2 (0.21)	3.2 (0.04)	53.3 (0.29)	0.2 (0.03)	2.8 (0.12)	0.06 (8E−4)	0.1 (0.02)
HMA[d]			7.5 (0.24)	4.5 (0.07)	29.7 (1.72)	nq	9.5 (0.17)	0.5 (0.008)	1.4 (0.07)
HMB[d]			18.5 (0.43)	5.9 (0.06)	70.7 (3.71)	nq	22.1 (0.09)	0.1 (0.01)	0.6 (0.02)
HMC[d]			3.4 (0.42)	1.0 (0.01)	31.9 (2.35)	0.07 (0.02)	0.1 (0.02)	0.1 (0.02)	0.1 (0.01)
min value			0.3	nq	6.1	nq	0.1	0.04	0.03
max value	159.9	60.5	34.7	21.8	100.6	3.3	25.9	0.8	1.4
mean	26.9	6.7	12.1		38.8		7.5	0.3	0.3
SD	37.32	16.31	10.83		28.07		7.07	0.23	0.34

[a]Values were expressed as mean (standard deviation) of three assays for each sample. nq-not quantified. [b] 1, 2, 3: these numbers represent different blends of quince jam samples from the same manufacture industry. [c] Arbutin and gallic acid were determined by the simplified technique. [d] HMA, HMB, HMC: homemade quince jams from different origins.

Table 2. Procyanidin Composition of Quince Jams (mg Procyanidin/kg Jam) Obtained by Amberlite XAD-2 Extraction[a]

samples[b]	procyanidinB3[c] (RT 8m25s)	procyanidin u[d] (RT 22m12s)	procyanidin v[d] (RT 24m27s)	procyanidin x[d] (RT 26m06s)	procyanidin y[d] (RT 26m33s)
A1	18.5 (3.90)	169.5 (15.83)	5.6 (0.75)	2.8 (0.28)	16.3 (1.23)
A2	nq	83.5 (8.63)	5.7 (0.95)	265.9 (19.92)	11.9 (1.51)
A3	nq	13.0 (0.28)	139.2 (2.37)	6.3 (0.12)	19.6 (0.83)
B1	76.2 (8.02)	240.0 (11.00)	22.4 (0.56)	13.0 (2.29)	605.8 (37.73)
B2	nq	36.3 (8.10)	75.2 (1.31)	3.2 (0.34)	69.9 (7.44)
B3	nq	31.1 (7.18)	115.6 (3.95)	4.1 (0.37)	13.7 (1.29)
C1	243.6 (1.19)	344.4 (35.10)	7.5 (1.98)	nq	2464.9 (639.48)
C2	318.6 (31.72)	404.6 (15.48)	16.8 (0.51)	26.3 (2.63)	35.5 (2.41)
C3	nq	20.2 (2.28)	44.6 (5.04)	439.7 (13.07)	5.1 (0.28)
D1	215.4 (31.78)	609.6 (34.38)	25.3 (1.06)	178.9 (10.19)	nq
D2	nq	210.9 (12.86)	nq	7.9 (0.56)	21.1 (1.01)
E1	234.7 (14.00)	1086.7 (74.60)	14.4 (1.53)	96.9 (86.41)	747.9 (123.14)
E2	nq	458.2 (10.97)	nq	nq	36.9 (1.09)
E3	nq	2.3 (0.07)	6.9 (0.27)	311.5 (36.13)	2.0 (0.09)
F1	nq	13.6 (0.28)	105.1 (1.80)	8.8 (0.56)	17.1 (0.10)
F2	nq	34.7 (2.26)	96.3 (7.70)	8.7 (0.24)	1.9 (0.36)
F3	nq	nq	266.7 (0.33)	13.6 (0.78)	24.5 (1.29)
HMA[e]	nq	295.8 (8.15)	24.4 (7.84)	3.5 (0.33)	25.4 (3.36)
HMB[e]	319.9 (81.28)	13.3 (9.63)	19.2 (5.11)	260.2 (25.68)	32.3 (0.61)
HMC[e]	nq	3.3 (0.30)	395.4 (4.23)	17.7 (0.07)	38.8 (0.35)

[a] Values were expressed as mean (standard deviation) of three assays for each sample. nq-not quantified. [b] 1, 2, 3: these numbers represent different blends of quince jam samples from the same manufacture industry. [c] Procyanidin B3 was determined by the simplified technique. [d] u, v, x, y: unidentified characteristic procyanidins of quince jam which are under study. [e] HMA, HMB, HMC: homemade quince jams from different origins.

range with the library of spectra previously compiled by the authors. Peak purity was checked by means of the Gilson 160 SpectraViewer Software Contrast Facilities.

Phenolics quantification was achieved by the absorbance recorded in the chromatograms relative to external standards of phenolic compounds with detection at 350 nm for 3-, 4-, and 5-*O*-caffeoylquinic acids, quercetin 3-galactoside, quercetin 3-xyloside, quercetin 3-rhamnoside and rutin and 280 nm for the others. The concentration of procyanidin polymers was expressed as procyanidin B3.

RESULTS AND DISCUSSION

Under the conditions described in the Materials and Methods, the retention times obtained were those indicated in Tables 1−3. The repeatability of the method was high, with respect to both retention times and peak areas.

With the Amberlite XAD-2 cleaning step, as a general rule, the extract had an higher amount of each phenolic compound. When this extractive method is used arbutin and procyanidin B3 presented a lower recover rate (data not shown), which could be due to the polarity of these compounds, allowing its elution with the sugars and other polar compounds. So the simplified technique is needed for the quantification of arbutin, in adulterated quince jams, and procyanidin B3 (Andrade et al., 1998) (Tables 1 and 2).

For all quince jams, except for those adulterated, both extraction techniques led to the same phenolic profiles, composed of, at least, eight identified phenolic compounds, (procyanidin B3, 3-, 4-, and 5-*O*-caffeoylquinic acids, quercetin 3-galactoside, quercetin 3-xyloside, quercetin 3-rhamnoside, and rutin) (Figures 1 and 2, Tables 1 and 2). Quince jam profiles (Figure 2) also showed the presence of several unidentified compounds with identical UV spectra when recorded with a diode array detector (identical shape and maximum at 269.3 nm). The possibility of being glycosides of procyanidin polymers is not excluded in accordance with the Porter et al. (1985) studies, their chromatographic behavior, and their UV spectra. Some samples also presented gallic acid (Figure 3), which is detected only in the

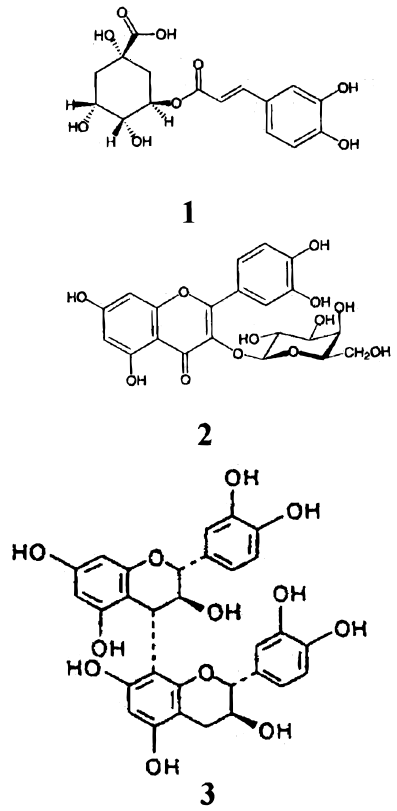

Figure 1. Chemical structure of major identified compounds in quince jam: (1) 5-*O*-caffeoylquinic acid; (2) quercetin 3-galactoside; (3) procyanidin B3.

extracts obtained when the simplified technique with methanol was used. When the Amberlite XAD-2 step is used this compound disappears completely from the extract, probably because it is removed by the water as happens with arbutin and procyanidin B3.

Most of the samples analyzed (simplified technique) showed an HPLC profile with arbutin (Figure 4), which suggests adulteration with pear. None of the quince jams presented the dihydrochalcones phloretin 2′-xylosylglucoside and phloretin 2′-glucoside, considered the

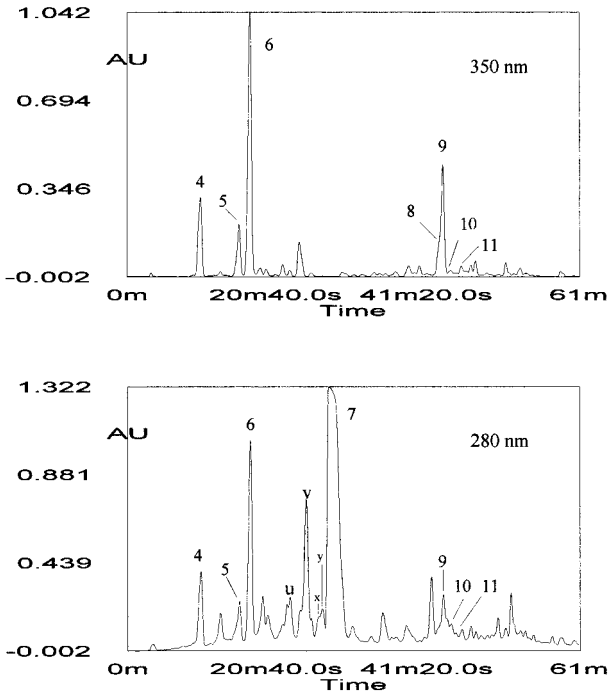

Figure 2. HPLC profile of quince jam phenolics obtained by Amberlite XAD-2 extraction: (4) 3-*O*-caffeoylquinic acid; (5) 4-*O*-caffeoylquinic acid; (6) 5-*O*-caffeoylquinic acid; (7) sodium benzoate; (8) rutin; (9) quercetin 3-galactoside; (10) quercetin 3-xyloside; (11) quercetin 3-rhamnoside; u, v, x, and y,-unidentified characteristic procyanidins of quince jam.

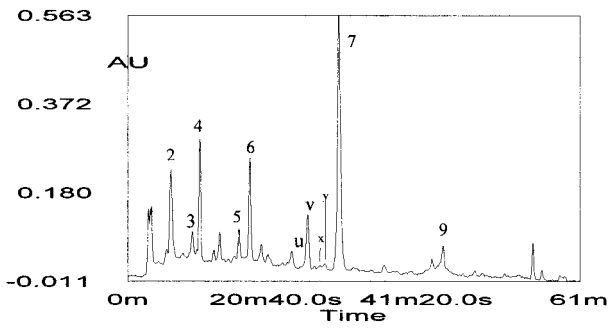

Figure 3. HPLC profile of quince jam phenolics obtained by simple extraction with methanol. Detection at 280 nm: (2) gallic acid; (3) procyanidin B3; (4) 3-*O*-caffeoylquinic acid; (5) 4-*O*-caffeoylquinic acid; (6) 5-*O*-caffeoylquinic acid; (7) sodium benzoate; (9) quercetin 3-galactoside; u, v, x, and y, unidentified characteristic procyanidins of quince jam.

chemical markers of apple (Dick et al., 1987; Oleszek et al., 1988; Burda et al., 1990).

On the quantitative level (Table 1 and Table 2), either in the homemade quince jams as in the commercial ones, the major compound is one of the unidentified procyanidins. In addition, different blends of quince jam samples from the same manufacture industry presented different composition; this can be due to a deficiency in the control of the manufacturing cooking process of the quince jam, namely the time and cooking temperature.

In a general way, in what concerns the phenolic acids and flavonoidic heterosides, 5-O-caffeoylquinic acid and quercetin-3-galactoside (Figure 1), respectively, are the compounds present in higher amounts.

On the HPLC profiles obtained at 280 nm (Figure 3) it was also possible to observe a peak corresponding to sodium benzoate, the most popular preservative agent

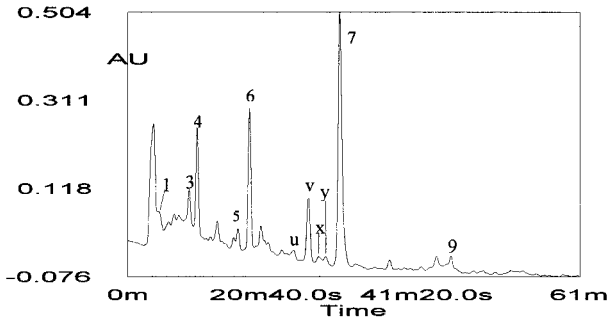

Figure 4. HPLC profile of adulterated quince jam phenolics obtained by simple extraction with methanol. Detection at 280 nm: (1) arbutin; (3) procyanidin B3; (4) 3-*O*-caffeoylquinic acid; (5) 4-*O*-caffeoylquinic acid; (6) 5-*O*-caffeoylquinic acid; (7) sodium benzoate; (9) quercetin 3-galactoside; u, v, x, and y, unidentified characteristic procyanidins of quince jam.

Table 3. Sodium Benzoate Composition of Commercial Quince Jams (g/100 g Jam) Obtained by the Simplified Technique[a]

samples[b]	sodium benzoate (RT 27m45s)
A1	0.03 (0.001)
A2	0.03 (0.014)
A3	0.01 (0.001)
B1	0.07 (0.003)
B2	0.07 (0.002)
B3	0.05 (0.003)
C1	0.12 (0.003)
C2	0.13 (0.005)
C3	0.02 (0.001)
D1	1.25 (0.025)
D2	0.83 (0.086)
E1	0.03 (0.003)
E2	0.03 (0.001)
E3	0.04 (0.004)
F1	0.07 (0.010)
F2	0.07 (0.001)
F3	0.07 (0.006)
min value	0.01
max value	1.25
mean	0.17
SD	0.337

[a] Values were expressed as mean (standard deviation) of three assays for each sample. [b] 1, 2, 3: these numbers represent different blends of quince jam samples from the same manufacture industry.

used by industry to preserve jams against yeasts and molds. The quantification of this compound (Table 3) was done by the simplified technique, according to Andrade et al. (1999) studies, once the recover rate of sodium benzoate is higher than with the Amberlite XAD-2 extraction. All the samples presented values of sodium benzoate below the maximum permitted (in quince jam, sodium benzoate can be found until 1.5 g/Kg of final product (Portaria no. 497/92)), with the exception of samples D1 and D2. This could be explained by bearing in mind that this quince jam is a light product, so the sugar quantity is lower than in the other quince jams and a higher amount of preservative is needed.

In conclusion, this study suggests that the technique presented herein is quite useful for the simultaneous analysis of phenolic compounds and sodium benzoate in commercial quince jam samples, allowing the detection of apple and/or pear in these jams and to verify if sodium benzoate values are within the limits established by Portuguese legislation.

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