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Study of the Organic Acids Composition of Quince (*Cydonia oblonga* Miller) Fruit and Jam

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The organic acids present in several samples of quince fruit (pulp and peel) and quince jam (homemade and industrially manufactured) were analyzed by HPLC. The sample preparation was simple, involving only extraction with methanol (40 °C) and filtration through a Sep-pack C18 cartridge. The chromatographic separation was achieved using an ion exclusion column, Nucleogel Ion 300 OA (300 × 7.7 mm), in conjunction with a column heating device at 30 °C. An isocratic elution with H_2SO_4 0.01 N as the mobile phase, with a flow rate of 0.1 mL/min, and UV detection at 214 nm were used. These analyses showed that all samples presented a similar profile composed of at least six identified organic acids: citric, ascorbic, malic, quinic, shikimic, and fumaric acids. Several samples also contained oxalic acid. This study suggests that the organic acids levels and ratios may be useful for the determination of percent fruit content of quince jams. The citric acid value can also be used in the differentiation of the type of manufacture of the commercial quince jams (homemade or industrially manufactured).

KEYWORDS: ***Cydonia oblonga* Miller; quince fruit; quince jam; HPLC−UV; organic acids**

INTRODUCTION

Quince is the fruit of a deciduous tree of the *Rosaceae* family, *Cydonia oblonga* Miller. When ripe, quince fruits impart an agreeable, long-lasting, and powerful flavor. As they are not edible unprocessed because of their very hard, tough, and fibrous consistency, they are often used for preparing jam (*1*). Quince jam, designated "marmelada", is a compote with great tradition in Portugal. Because quince is a seasonal fruit, its homemade jam is prepared during September/October by boiling a mixture of sugar and quince fruits, normally in the proportion of 50:50, until the appropriate consistency is reached (usually 65−72 °Brix) (*2*). The industrially manufactured quince jam is prepared with quince puree, sugar, and additives (preservatives such as benzoic and sorbic acids; antioxidants such as ascorbic acid; and acidity regulators such as citric and tartaric acids; etc.).

The nature and the concentration of organic acids are important factors influencing the organoleptic properties of fruit and fruit products (*3*). Accurate knowledge of organic acid levels (and ratios) is sometimes useful to determine the percent fruit content of fruit products, and also to detect adulteration in this food class (*4*). Acids are components of fruits that have a lower susceptibility to change during processing and storage than other components such as pigments and flavor compounds. This relative stability offers a practical advantage for using an organic acids profile as an index of authenticity in fruit products (*5*, *6*). However, separation and accurate quantification of individual organic acids in these products have been considered difficult because of their structural similarities and lack of distinctive spectral properties (*3*). Furthermore, most of the organic acids of interest in fruit products are weak acids with similar pK_a values. Because of these similarities, their chromatographic behaviors are identical, and therefore they cannot be separated quantitatively if one of the acids is present in relatively large amounts (*3*).

For quince fruit and its derivatives, few studies have been developed. As far as we know, there is no study about organic acids composition of quince fruit and/or its jam. So, the work herein represents a contribution for the definition of the organic acids profile of the pulp and peel from this fruit and its jam. With this purpose, samples of quince fruit from seven different geographic origins from Portugal, as well as twenty commercial quince jam samples (four homemade and sixteen industrially manufactured) were analyzed. A quince jam was also prepared with pulps of fruits from one of the geographic origins analyzed to check the usefulness of organic acids composition in the determination of the percent fruit content of quince jams.

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). All fruits were separated into pulp and peel. Each part of the fruit was cut into thin slices and freeze-dried.

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A quince jam was prepared in the laboratory by boiling quince pulps from Amarante with sugar (in the proportion of 50:50) during approximately 60 min.

Twenty commercial quince jam samples, randomly purchased on the Portuguese market, were assayed, and these included four that were homemade (samples A−D) and sixteen that were industrially manufactured (samples E−T).

Standards. The standards were from Sigma (St. Louis, MO) and from Extrasynthése (Genay, France). Methanol and hydrochloric acid were obtained from Merck (Darmstadt, Germany), and sulfuric acid was obtained from Pronalab (Lisboa, Portugal). 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).

Extraction of Organic Acids. Each sample (ca. 3 g for quince jams and 1 g for quince pulps and peels) was thoroughly mixed with methanol (10 × 50 mL) or acid water (pH = 2 with HCl). Two different temperatures of extraction were assayed: ambient and 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. 50 mL). The aqueous solutions obtained were then passed through an ISOLUTE C18 (NEC) column, previously conditioned with 30 mL of methanol and 70 mL of acid water (pH 2 with HCl). The aqueous extracts were evaporated to dryness under reduced pressure (40 °C) and redissolved in sulfuric acid 0.01 N (3 mL), and 20-μL samples were analyzed by HPLC.

HPLC Analysis of Organic Acids. The separation of organic acids was achieved with an analytical HPLC unit (Gilson), using an ion exclusion column (Nucleogel Ion 300 OA; 300 × 7.7 mm) or a Spherisorb ODS2 column (25.0 × 0.46 cm; 5μm, particle size) in conjunction with a column heating device at 25, 30, 40, 50, 60, and 70 °C. Elution was carried out isocratically with sulfuric acid (0.01, 0.005, and 0.0025 N) as the mobile phase. The following flow rates were assayed: 0.1, 0.2, 0.3, 0.4, and 0.5 mL/min. 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.

Statistics. Data are presented as the mean ± standard deviation. For statistical evaluation of citric and ascorbic acids contents from industrially manufactured and homemade quince jams a one-way analysis of variance (ANOVA) was used. Means evaluated were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

To optimize the HPLC conditions for analysis of organic acids from quince fruit and its jam, an artificial mixture was prepared containing thirteen acids previously reported from other fruits (especially those from pear and apple which also belong to the *Rosaceae* family) (*3*−*14*): oxalic, *cis*-aconitic, citric, ascorbic, α-ketoglutaric, pyruvic, malic, quinic, succinic, lactic, shikimic, fumaric, and tartaric, as there are no studies on the organic acids composition of quince. To choose the best conditions of analysis, several methodologies of separation were tried, and the best results were obtained using an ion exclusion Nucleogel Ion 300 OA (300 × 7.7 mm) column, in conjunction with a column heating device at 30 °C, and isocratic elution with sulfuric acid 0.01 N, at a 0.1 mL/min flow rate. This chromatographic procedure has the advantage of allowing the detection of many of the organic acids of interest in quince fruit and its jam without precolumn derivatization.

From the assayed extraction conditions, higher yields were obtained using methanol at 40 °C. The filtration through a Sep-pack C18 (NEC) cartridge eliminates interference compounds, such as phenolics, that are retained by the sorbent.

All samples presented a similar profile composed of, at least, six identified organic acids: citric, ascorbic, malic, quinic, shikimic and fumaric acids. Several of them also contained oxalic acid. None of the samples revealed the presence of *cis*-aconitic, α-ketoglutaric, pyruvic, succinic, lactic, or tartaric acids.

Under the assay conditions described, a linear relationship between the concentration of oxalic, citric, ascorbic, malic, shikimic, and fumaric acids and the UV absorbance at 214 nm was obtained. The correlation coefficient for the standard curves invariably exceeded 0.99 for all organic acids. The calibration curves were obtained by triplicate determinations of each of the calibration standards, and the peak area values (arbitrary units) were plotted as average values. The relative percent average deviations of triplicates were less than 2% in all cases. The average regression equation for oxalic, citric, ascorbic, malic, shikimic, and fumaric acids were $y = 5.27 \times 10^8x$, $y = 7.90 \times 10^7x$, $y = 1.33 \times 10^7x$, $y = 5.95 \times 10^7x$, $y = 4.84 \times 10^9x$, and $y = 1.05 \times 10^{10}x$, respectively.

The detection limit values were calculated as the concentration corresponding to three times the standard deviation of the background noise, and the values obtained ranged from 0.01 to 1.67 μg/mL. The precision of the analytical method was evaluated by measuring the peak chromatographic area of organic acids six times on the same sample. The analytical method is precise: the coefficients of variation of organic acids were between 1.52 and 5.36%.

To study the recovery of the procedure, one quince pulp, one peel, and one jam sample were added to known quantities of oxalic, citric, ascorbic, malic, shikimic, and fumaric acids. The samples were analyzed in triplicate before and after the additions. Recovery values were high, between 82.9 and 112.1%, which demonstrates the effectiveness of the extraction and the accuracy of the method.

Quince Fruits. As previously stated, all quince pulps and peels presented citric, ascorbic, malic, quinic, shikimic, and fumaric acids (**Table 1**). Several samples also contained oxalic acid. The sum of all quantified acids ranged from ca. 7 to 14 g/kg, either in pulps or in peels. In some cases (Amarante, Vila Real, Bragança, and Pinhel) the amount of acids was higher in pulps than in the corresponding peels; in other cases the differences were very small, and even in one case (Baião) the peels were richer than the pulps. As can be seen in **Table 1**, the total amounts of organic acids were related to the quince fruit geographical origin. However, other factors, such as cultural practices and degree of maturation, may have caused the observed differences.

Despite this variability, there are features common to all analyzed samples. In all cases the sum of malic acid plus quinic acid represented 96 to 99% of the total acid content in pulps and 93 to 98% in peels. That fact means that all other acids were present in very small amounts, usually lower than 1%, with the exception of citric acid which ranged from traces to 6%.

Quince Jams. To test the usefulness of the knowledge of organic acids levels and their ratios in the determination of the percent fruit content, and to study the behavior of the acids during processing, a quince jam was prepared in the laboratory by boiling pulps of fruits from Amarante and sugar (50:50). On comparing the total acids amount of this jam with that of the used pulp, it can be seen that it corresponds to approximately 52%, which is very similar to the percent fruit content utilized (50%). Moreover, the ratios among the acids stayed roughly

Table 1. Organic Acids Composition of Pulp and Peel from Quince Fruit[a]

	organic acids (mg/kg)						
geographic origin	oxalic acid (RT 39.56 min)	citric acid (RT 53.83 min)	ascorbic acid (RT 57.50 min)	malic + quinic acids (RTs 64.93, 67.57 min)	shikimic acid (RT 85.48 min)	fumaric acid (RT 117.34 min)	Σ
				pulp			
Amarante	9.1 (0.16)	179.9 (1.69)	31.2 (1.32)	10302.2 (132.96)	14.2 (0.16)	tr	10536.6
Baião	3.0 (0.08)	tr	158.1 (5.43)	6725.2 (135.81)	15.0 (0.13)	0.1 (0.01)	6901.4
Vila Real	tr	240.8 (2.03)	38.5 (0.94)	10985.7 (64.66)	19.3 (0.06)	0.1 (0.01)	11284.4
Bragança	nd	14.7 (0.41)	116.8 (5.38)	12642.9 (133.46)	11.1 (0.01)	0.9 (0.01)	12786.4
Covilhã	5.0 (0.14)	558.8 (12.69)	15.8 (2.08)	13358.3 (310.63)	24.1 (0.29)	0.1 (0.01)	13962.1
Viseu	nd	253.4 (2.33)	37.9 (0.79)	9380.2 (64.46)	18.5 (0.01)	0.1 (0.01)	9690.1
Pinhel	6.8 (0.40)	55.6 (0.92)	45.7 (1.84)	14057.7 (100.92)	19.8 (0.03)	0.2 (0.01)	14185.8
min	nd	tr	15.8	6725.2	11.1	tr	6901.4
max	9.1	558.8	158.1	14057.7	24.1	0.9	14185.8
mean	3.4	186.2	63.4	11064.6	17.4	0.2	11335.3
SD	3.68	194.72	52.77	2548.39	4.30	0.31	2586.43
				peel			
Amarante	10.9 (0.23)	66.0 (1.83)	122.2 (11.57)	8771.6 (106.21)	17.1 (0.10)	tr	8987.8
Baião	nd	269.5 (3.97)	43.8 (3.49)	13174.1 (266.97)	23.1 (0.36)	0.6 (0.02)	13511.1
Vila Real	12.1 (0.55)	92.5 (6.08)	150.2 (2.03)	8873.7 (346.21)	26.0 (1.03)	5.9 (0.02)	9160.4
Bragança	tr	47.9 (3.23)	124.4 (2.09)	7565.9 (338.33)	18.6 (0.15)	1.1 (0.01)	7757.9
Covilhã	tr	140.2 (0.92)	73.8 (3.67)	13727.8 (1.29)	32.7 (0.75)	0.1 (0.01)	13974.6
Viseu	nd	204.0 (5.60)	126.7 (1.75)	10410.9 (175.53)	27.2 (0.01)	0.5 (0.02)	10769.3
Pinhel	nd	792.6 (99.67)	76.5 (1.57)	12606.6 (438.36)	20.7 (0.32)	0.4 (0.05)	13496.8
min	nd	47.9	43.8	7565.9	17.1	tr	7757.9
max	12.1	792.6	150.2	13727.8	32.7	5.9	13974.6
mean	3.3	230.4	102.5	10732.9	23.6	1.2	11094.0
SD	5.62	260.06	38.02	2445.29	5.44	2.09	2560.06

[a] Values are expressed as mean (standard deviation) of three determinations; tr, traces; nd, not detected; Σ, sum of the determined organic acids; RT, retention time.

Table 2. Organic Acids Composition of Quince Pulp from Amarante and Its Jam[a]

	organic acids (mg/kg)						
samples	oxalic acid (RT 39.56 min)	citric acid (RT 53.83 min)	ascorbic acid (RT 57.50 min)	malic + quinic acids (RTs 64.93, 67.57 min)	shikimic acid (RT 85.48 min)	fumaric acid (RT 117.34 min)	Σ
pulp	9.1 (0.16)	179.9 (1.69)	31.2 (1.32)	10302.2 (132.96)	14.2 (0.16)	tr	10536.6
jam	7.0 (0.41)	91.4 (0.22)	14.8 (0.88)	5414.7 (111.86)	5.3 (0.06)	tr	5533.2

[a] Values are expressed as mean (standard deviation) of three determinations; tr, traces; Σ, sum of the determined organic acids; RT, retention time.

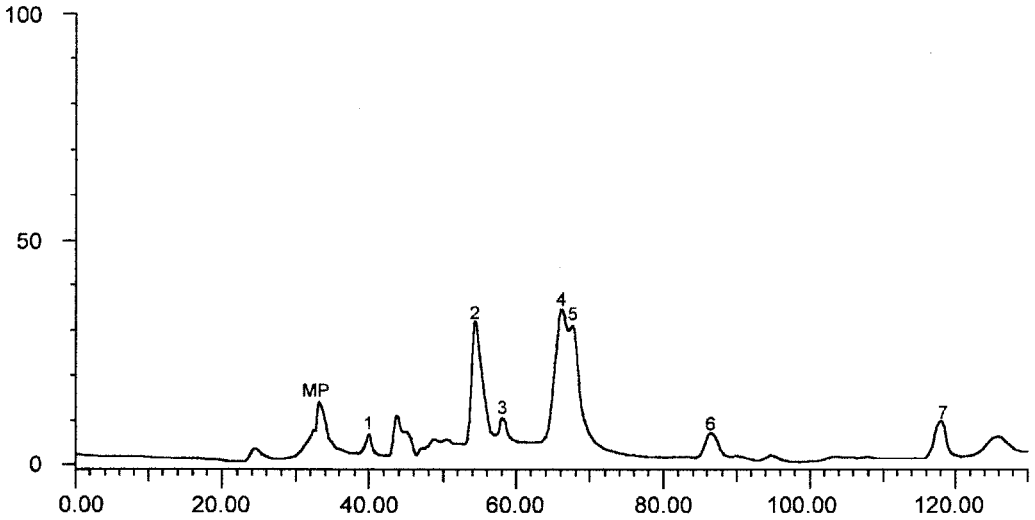

Figure 1. HPLC organic acids profile of a quince jam, detection at 214 nm: (MP) mobile phase; (1) oxalic acid; (2) citric acid; (3) ascorbic acid; (4) malic acid; (5) quinic acid; (6) shikimic acid; (7) fumaric acid.

constant, even for ascorbic acid, the most thermolabile of the detected acids (**Table 2**).

Twenty commercial quince jam samples were analyzed by the proposed technique, and **Figure 1** shows a typical HPLC profile of an industrially manufactured quince jam sample. In a general way, as it happened with quince fruits, either in the homemade quince jams (samples A−D) or in the industrially manufactured ones (samples E−T), the major peaks in the chromatograms correspond to the sum of malic and quinic acids (**Table 3**).

Table 3. Organic Acids Composition of Commercial Quince Jam Samples[a]

	organic acids (mg/kg)						
sample	oxalic acid (RT 39.56 min)	citric acid (RT 53.83 min)	ascorbic acid (RT 57.50 min)	malic + quinic acids (RTs 64.93, 67.57 min)	shikimic acid (RT 85.48 min)	fumaric acid (RT 117.34 min)	Σ
A[b]	tr	4.6 (0.11)	101.0 (4.36)	5303.3 (81.88)	7.5 (0.16)	1.6 (0.20)	5418.0
B[b]	nd	3.7 (0.13)	158.8 (26.10)	5697.8 (498.59)	6.2 (0.21)	1.3 (0.07)	5867.8
C[b]	nd	tr	426.0 (48.52)	2280.9 (69.55)	5.7 (0.45)	0.1 (0.01)	2712.6
D[b]	tr	tr	120.6 (3.64)	2385.4 (63.84)	8.1 (0.62)	tr	2514.1
E[c]	37.3 (2.00)	1758.1 (26.67)	208.1 (5.35)	976.2 (51.97)	7.8 (0.36)	0.1 (0.01)	2987.6
F[c]	1.4 (0.02)	876.2 (6.79)	207.9 (2.79)	3921.5 (38.48)	5.5 (0.01)	0.1 (0.02)	5012.5
G[c]	3.0 (0.10)	1425.6 (22.45)	68.3 (10.29)	2931.1 (96.67)	0.9 (0.06)	0.1 (0.02)	4428.9
H[c]	tr	710.1 (8.39)	103.0 (9.45)	4684.1 (59.43)	14.8 (0.01)	tr	5512.0
I[c]	2.3 (0.01)	944.3 (7.18)	153.1 (3.86)	5778.5 (236.08)	7.8 (0.13)	0.1 (0.01)	6886.0
J[c]	3.5 (0.05)	1184.5 (35.87)	793.7 (26.26)	4124.2 (169.83)	10.8 (0.03)	1.2 (0.21)	6117.9
K[c]	tr	825.5 (45.99)	115.8 (9.68)	6539.3 (558.15)	8.9 (0.01)	0.6 (0.04)	7490.1
L[c]	tr	817.7 (4.46)	84.3 (4.13)	2194.2 (240.64)	13.2 (0.13)	0.1 (0.01)	3109.4
M[c]	nd	1150.1 (64.08)	176.3 (1.54)	8077.6 (25.33)	13.9 (0.05)	2.1 (0.01)	9420.0
N[c]	1.8 (0.12)	229.8 (9.09)	130.3 (7.86)	5464.2 (91.19)	9.1 (0.01)	1.2 (0.05)	5836.4
O[c]	2.6 (0.08)	1089.4 (15.82)	165.2 (24.43)	10059.7 (27.74)	14.3 (0.02)	0.2 (0.01)	11331.4
P[c]	12.1 (0.07)	3096.6 (17.33)	tr	9061.5 (564.30)	7.2 (0.16)	tr	12177.5
Q[c]	nd	210.4 (2.07)	144.0 (17.34)	11684.2 (284.11)	13.2 (0.24)	tr	12051.8
R[c]	5.7 (0.12)	1849.8 (82.50)	tr	2045.0 (96.59)	6.9 (0.05)	0.1 (0.01)	3907.4
S[c]	tr	993.5 (68.38)	225.9 (20.18)	3185.9 (117.52)	30.5 (0.16)	0.1 (0.01)	4435.8
T[c]	1.5 (0.00)	3860.9 (65.28)	107.3 (10.60)	3812.7 (37.98)	9.2 (0.09)	1.7 (0.27)	7793.2
min	nd	tr	tr	976.2	0.9	tr	2514.1
max	37.3	3860.9	793.7	11684.2	30.5	2.1	12177.5
mean	3.6	1051.5	174.5	5010.4	10.1	0.5	6250.5
SD	8.45	1011.41	171.21	2884.17	5.95	0.70	3001.44

[a] Values are expressed as mean (standard deviation) of three determinations. tr, traces; nd, not detected; SD, standard deviation; Σ, sum of the determined organic acids; RT, retention time. [b] Homemade quince jam samples. [c] Industrially manufactured quince jam samples.

The homemade samples maintained a profile very similar to that observed for quince fruits, except for jam C, where ascorbic acid reached ca. 16%. This could be due to an addition of ascorbic acid to this quince jam, although the fact was not mentioned on the label.

In the industrially manufactured jams the total acid content ranged from 3 to 12 g/kg, but the ratio among the acids was altered. This is explained by the addition of citric and ascorbic acids (as mentioned on the label, although the amounts were not provided) as acidity regulator and antioxidant, respectively.

Regarding the citric acid contents, there are significant differences between the two manufacture types ($F = 7.12$, $p = 0.05$): in the homemade quince jams this acid is presented in very low content (mean value of 2.1 mg/kg; $n = 4$), when compared to industrially manufactured jams (mean value of 1313.9 mg/kg; $n = 16$). So, it is possible to distinguish the quince jams type of manufacture (industrial or homemade) by this parameter.

The difference between the mean value of ascorbic acid content from homemade (201.6 mg/kg; $n = 4$) and industrially manufactured quince jams (167.7 mg/kg; $n = 16$) was not significant ($p > 0.05$).

All the organic acids herein detected in quince were already described in apple and pear: two fruits also belonging to the same botanical family (*Rosaceae*) and frequently used to adulterate quince jams. So, contrarily to what happens with the phenolic profile (*2*), it is not possible to detect any of these falsifications by the qualitative analysis of organic acids. The detection of such adulteration by the analysis of the acids ratios does not seem very feasible: first, because, as far as we know, there is no accurate knowledge about the acids ratios in these fruits; and second, because, as already mentioned, the law allows the addition of citric and ascorbic acids. Despite this, the study of the organic acids ratios allows an approximate evaluation of the amounts of the added acids. When analyzing **Table 3** under this point of view, we are induced to suspect that some of the jams, namely sample E, were adulterated. This sample has a very low content of malic plus quinic acids and a high amount of citric acid. Maybe this high addition of citric acid was intended to mask a low amount of quince, while maintaining the pH within the legal values (between 2.8 and 3.5) (*15*). So, knowledge of the composition of quince fruit and jam in terms of organic acids may help in the quality control of those products.

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