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Free Amino Acid Composition of Quince (*Cydonia oblonga* Miller) Fruit (Pulp and Peel) and Jam

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Twenty-one free amino acids present in several samples of quince fruit (pulp and peel) and quince jam (homemade and industrially manufactured) were analyzed by GC/FID. The analyses showed some differences between quince pulps and peels. Generally, the highest content in total free amino acids and in glycine was found in peels. As a general rule, the three major free amino acids detected in pulps were aspartic acid, asparagine, and hydroxyproline. For quince peels, usually, the three most abundant amino acids were glycine, aspartic acid, and asparagine. Similarly, for quince jams the most important free amino acids were aspartic acid, asparagine, and glycine or hydroxyproline. This study suggests that the free amino acid analysis can be useful for the evaluation of quince jam authenticity. It seems that glycine percentage can be used for the detection of quince peel addition while high alanine content can be related to pear addition.

KEYWORDS: ***Cydonia oblonga*** **Miller; quince fruit; quince jam; GC/FID; free amino acids**

INTRODUCTION

Besides proteins, plants and fruits possess amino acids in their free form, which mostly show a typical pattern (*1*). Determination of this pattern provides some information about possible adulteration or falsification of their derivatives, namely, fruit juices, wines, and jams (*1, 2*).

Amino acids, a class of biologically active compounds present in food and beverages, are important for human nutrition and affect the quality of foods including taste, aroma, and color (*1*). Among different substances that constitute fruits and vegetables, amino acids are becoming increasingly important and, for various reasons, their analytical determination is becoming more necessary (*3*). First, the concentration of amino acids in the fruit varies significantly as a result of metabolic changes during growth and ripening, and this can be exploited to determine the optimum ripening time (*3*). Second, amino acid profiles vary from one species to another and among fruits of the same type but with different origin, so they can probably be used to characterize fruit products (*3, 4*). Finally, amino acids can influence the quality of fruit-derived products because they take an active part in the Maillard reaction and in browning processes after the enzymatic oxidation of polyphenols, which determine the sensorial quality of such products like juices and jams (*3*).

Quince is the fruit of a deciduous tree of the *Rosaceae* family, *Cydonia oblonga* Miller. Because of its hardness, acidity, and astringency, it is not edible unprocessed; nevertheless, it is often used to prepare jam. Quince jam, either homemade or industrially manufactured, is obtained by boiling a mixture of sugar and quince puree until convenient consistency is obtained (usually to reach 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.) because of their low cost and similar texture and rheological properties. Once the stronger odor of quince masks the sweet flavors of both fruits, sensory evaluation cannot be used to detect their presence (*5*).

Few chemical studies have been developed for quince fruit and its derivatives. In 1979 and 1986, the volatile constituents of quince fruit were analyzed by GC/MS (*6, 7*). Later, the usefulness of phenolic compounds in the determination of genuineness of quince puree (*8*), jam (*9, 10*), and jelly (*5*) has been reported. Glucosides of procyanidin polymers have been previously identified in this fruit (*11, 12*). Recently, it was possible to discriminate quince pulp and peel by the analysis of phenolic compounds in quince fruit (*13*). In 2002, an HPLC/UV method was developed for the determination of organic acids in quince fruit and its jam (*14*). More recently, a GC/FID method was developed for the determination of free amino acids in quince fruit and jam (*15*). The referred method was then applied to the analysis of samples of quince fruit (pulp and peel) from seven different geographic origins from Portugal, as well as 20 commercial quince jam samples (4 homemade and 16 industrially manufactured).

MATERIALS AND METHODS

Samples. Healthy quince fruit samples were collected in different places in Northern (Amarante, Baião, Vila Real, and Bragança) and

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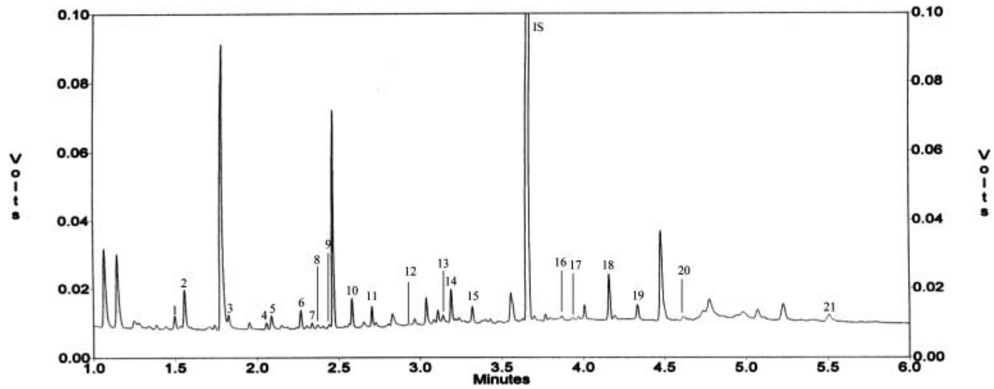

Figure 1. Free amino acids profile of quince jam: (1) alanine, (2) glycine, (3) valine, (4) leucine, (5) isoleucine, (6) proline, (7) threonine, (8) serine, (9) glutamic acid, (10) asparagine, (11) aspartic acid, (12) methionine, (13) hydroxyproline, (14) phenylalanine, (15) cysteine, (IS) internal standard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

Table 1. Retention Times of the Amino Acids ($n = 3$)

code	amino acid	retention time[a] (min)	SD (min)
1	alanine	1.50	0.001
2	glycine	1.59	0.001
3	valine	1.83	0.001
4	leucine	2.06	0.001
5	isoleucine	2.09	0.001
6	proline	2.27	0.002
7	threonine	2.34	0.001
8	serine	2.37	0.001
9	glutamic acid	2.44	0.001
10	asparagine	2.57	0.002
11	aspartic acid	2.72	0.002
12	methionine	2.93	0.003
13	hydroxyproline	3.12	0.003
14	phenylalanine	3.20	0.002
15	cysteine	3.29	0.002
IS	internal standard	3.66	0.002
16	glutamine	3.87	0.001
17	ornithine	3.94	0.003
18	lysine	4.17	0.002
19	histidine	4.33	0.004
20	tyrosine	4.64	0.003
21	tryptophan	5.52	0.004

[a] Mean of the retention times; SD, standard deviation.

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. Lyophilization was carried out using a Labconco 4.5 apparatus (Kansas City, MO).

Twenty commercial quince jam samples, including 4 homemade (samples A−D) and 16 industrially manufactured (samples E−T), randomly purchased on the Portuguese market were assayed.

Standards. The 21 L-amino acids and the internal standard (L-*p*-chlorophenylalanine) were all from Sigma (St. Louis, MO). Ethyl chloroformate (ECF) was from Aldrich (Steinheim, Germany), and pyridine was from Fluka (Neu-Ulm, Germany). All other chemicals were analytical grade from several suppliers.

Solid-Phase Extraction (SPE) Cartridges. The benzenesulfonic SCX Spe-ed SPE cartridges (200 mg, 3 mL) were obtained from Applied Separations (Allentown, PA).

Extraction of Free Amino Acids. The extraction was achieved as previously reported (*15*). Each sample (about 1.5 g for freeze-dried quince pulps and peels and 5 g for quince jams) was thoroughly mixed with 3 × 25 mL of acid water (pH 2.2 with 0.1 M HCl) at room temperature with magnetic stirring for 3 × 10 min. The extracts were gathered, filtered, and passed through a SCX cartridge previously conditioned with 10 mL of methanol and 10 mL of 5 mM HCl. The amino acids were eluted with a mixture of ammonia (4 M) and methanol

Table 2. Free Amino Acids Composition of Quince Pulps from Several Geographic Locations from Portugal (μg/kg) (Quantification by Internal Standard Technique)[a]

	geographic origin													
	Amarante		Baião		Vila Real		Bragança		Covilhã		Viseu		Pinhel	
amino acids	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
alanine	12.9	1.05	62.3	2.05	61.2	2.33	16.9	0.51	14.2	0.55	12.5	0.45	48.7	1.39
glycine	67.8	3.18	177.9	5.64	40.3	1.31	144.6	5.69	4.8	0.21	6.4	0.29	47.0	2.87
valine	13.8	0.64	47.9	1.21	13.3	0.60	19.0	0.62	4.5	0.16	5.8	0.25	21.1	1.09
leucine	4.8	0.16	15.8	1.08	3.8	0.15	4.9	0.31	2.1	0.13	1.3	0.05	6.7	0.11
isoleucine	13.7	0.26	70.2	2.97	14.2	0.06	12.8	0.62	11.6	0.72	6.1	0.18	21.1	0.57
proline	5.0	0.21	15.6	0.59	18.7	0.47	11.2	0.38	3.4	0.16	3.3	0.14	8.8	0.45
threonine	2.6	0.22	37.6	0.70	9.0	0.37	10.1	0.52	14.7	0.82	7.3	0.05	10.2	0.32
serine	9.8	0.76	77.9	6.49	39.4	2.26	19.2	0.22	51.6	0.69	14.5	0.22	33.1	1.64
glutamic acid	41.7	2.58	84.7	1.29	43.1	1.01	47.1	0.48	54.8	2.41	11.4	0.69	166.8	11.65
asparagine	90.7	2.72	130.6	3.56	307.1	6.33	188.7	5.86	54.7	0.37	40.3	2.12	121.4	3.55
aspartic acid	79.2	5.20	163.7	5.72	85.0	3.18	242.8	2.28	186.9	10.16	111.2	0.45	460.8	15.45
methionine	0.4	0.03	1.4	0.02	2.8	0.03	1.8	0.10	4.7	0.18	1.1	0.07	4.7	0.21
hydroxyproline	16.7	1.33	24.8	1.73	74.4	4.05	205.7	4.15	75.2	2.85	53.2	2.64	264.6	5.10
phenylalanine	8.0	0.09	13.3	0.05	4.9	0.06	3.0	0.17	1.2	0.02	0.6	0.02	14.2	0.52
cysteine	5.8	0.22	27.6	1.37	31.9	0.92	24.0	0.92	69.0	3.90	16.6	0.85	56.3	3.02
glutamine	2.9	0.21	20.3	0.40	2.4	0.18	35.0	2.32	6.7	0.15	8.2	0.16	28.8	0.30
ornithine	1.2	0.09	8.2	0.96	0.6	0.02	1.6	0.12	0.8	0.04	1.3	0.01	1.3	0.02
lysine	12.5	0.33	38.0	2.05	9.3	0.24	21.4	0.85	9.7	0.33	6.3	0.26	12.2	0.71
histidine	115.6	7.21	15.6	0.22	5.8	0.24	26.5	0.51	8.0	0.43	6.5	0.14	15.0	1.29
tyrosine	1.1	0.06	3.4	0.11	0.4	0.01	0.4	0.01	0.4	0.01	0.2	0.01	1.2	0.04
tryptophan	20.1	0.86	7.9	0.24	4.2	0.16	18.7	0.89	0.6	0.01	1.9	0.08	12.5	0.40
Σ	526.2		1044.6		771.9		1055.4		579.6		315.9		1356.6	

[a] Values are expressed as the mean of three determinations; SD, standard deviation; Σ, sum of the determined free amino acids.

Table 3. Free Amino Acids Composition of Quince Peels from Several Geographic Locations from Portugal (μg/kg) (Quantification by Internal Standard Technique)[a]

	geographic origin													
	Amarante		Baião		Vila Real		Bragança		Covilhã		Viseu		Pinhel	
amino acids	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
alanine	14.8	0.51	70.1	1.33	36.5	0.41	22.7	1.21	10.8	0.30	33.4	1.05	31.1	1.16
glycine	13.6	0.34	355.0	6.32	99.3	7.43	353.5	20.92	314.9	18.63	238.9	5.28	148.3	8.07
valine	13.6	0.45	63.0	1.21	6.4	0.27	27.3	0.93	12.4	0.43	24.4	0.64	16.0	0.68
leucine	5.0	0.08	18.3	0.28	1.3	0.03	5.7	0.22	1.4	0.07	2.2	0.11	3.0	0.04
isoleucine	15.4	0.70	125.4	2.20	22.3	0.63	55.3	1.74	35.2	0.78	24.8	0.60	19.0	0.66
proline	5.8	0.25	24.9	0.43	9.5	0.42	14.0	0.31	3.4	0.06	13.3	0.37	6.1	0.17
threonine	7.3	0.29	51.8	0.68	14.5	0.62	12.9	0.88	8.9	0.51	4.8	0.19	8.1	0.30
serine	14.7	1.40	145.6	2.10	28.1	1.55	29.5	1.97	21.1	1.28	30.8	1.30	38.2	0.52
glutamic acid	51.7	1.81	231.7	5.17	37.2	0.17	115.1	4.91	22.4	0.69	34.8	0.84	97.0	3.17
asparagine	139.1	12.46	112.6	2.15	101.9	3.65	207.0	4.26	40.4	0.63	177.6	6.32	72.8	1.95
aspartic acid	77.4	2.58	252.7	6.12	61.4	2.50	320.7	15.96	103.8	5.65	242.6	10.56	329.7	2.05
methionine	2.2	0.06	0.4	0.02	4.0	0.25	1.9	0.02	2.5	0.12	1.4	0.12	0.25	0.03
hydroxyproline	29.7	1.90	173.0	3.73	14.9	0.08	204.3	13.35	76.8	3.67	79.0	2.27	33.1	0.64
phenylalanine	11.0	0.25	13.7	0.26	2.9	0.10	6.0	0.39	0.8	0.05	1.1	0.04	4.2	0.17
cysteine	30.3	1.01	34.6	0.45	51.2	1.24	41.5	1.82	33.9	2.51	12.3	0.16	16.8	0.73
glutamine	21.9	0.97	27.2	0.63	2.8	0.01	12.2	0.31	1.5	0.04	19.4	0.56	1.7	0.12
ornithine	0.9	0.08	6.0	0.09	0.9	0.03	1.0	0.01	0.6	0.04	7.6	0.16	2.0	0.03
lysine	22.8	0.94	49.6	0.98	10.7	0.50	27.2	0.96	5.8	0.44	10.3	0.14	8.3	0.28
histidine	85.4	3.61	54.5	0.98	4.8	0.24	55.5	1.14	2.7	0.04	51.0	1.80	34.5	0.91
tyrosine	0.5	0.04	5.6	0.09	0.5	0.04	2.5	0.16	0.3	0.01	1.0	0.05	0.5	0.01
tryptophan	53.3	1.01	4.2	0.09	0.5	0.02	14.1	0.85	0.5	0.01	6.5	0.36	3.7	0.04
Σ	616.3		1819.9		511.7		1529.9		700.1		1017.3		874.1	

[a] Values are expressed as the mean of three determinations; SD, standard deviation; Σ, sum of the determined free amino acids.

(50:50 v/v) (3 × 500 μL). To each extract, an amount of 150 μL of L-*p*-chlorophenylalanine solution (10 μL/mL) (internal standard) was added. The obtained solutions were dried under N_2 stream and kept below 0 °C until derivatization.

Derivatization Procedure. The derivatization was achieved as previously reported (*15*−*19*). Each dried residue was dissolved in 60 μL of water and 40 μL of ethanol/pyridine (4:1), an amount of 5 μL of ECF was added, and the solution was vortex-mixed (3−5 s). Five minutes later, 150 μL of dichloromethane and ca. 0.01 g of NaCl were added and the vial was thoroughly shaken for extraction of the derivatives into the organic layer. This phase was transferred into a 200 μL insert adjustable to the liquid sampler vials. About 1.5 μL was injected into the gas chromatographic system.

GC Analysis. Separation of L-amino acids was achieved by gas chromatography, carried out with a Chrompack CP 9001 instrument (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector (FID) and an automatic liquid sampler (CP-9050, Chrompack). The injector was kept at 250 °C, and the detector was kept at 280 °C. The GC was equipped with an electronic pressure control, allowing programmable gas pressure during the chromatographic run. Helium as carrier gas was used with the following pressure program: increase from initial 50 (1 min hold) to 70 kPa at 4 min. A CP-Sil 19 CB (10 m × 0.25 mm i.d.) WCOT fused-silica capillary column (Varian) was used with the following temperature program: increase from 140 °C (1 min hold) to 280 °C at 40 °C/min (*15*).

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

Statistics. For statistical evaluation of total free amino acids and glycine content from quince pulps and peels, a one-way analysis of variance (ANOVA) was used. The mean values evaluated would be considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

All samples (quince fruits and jams) presented a similar qualitative profile with 21 free amino acids identified (**Figure 1**). The retention times obtained for amino acids are shown in **Table 1**.

Table 4. Free Amino Acids Composition of Homemade Quince Jams (μg/kg) (Quantification by Internal Standard Technique)[a]

	quince jams							
	A		B		C		D	
amino acids	mean	SD	mean	SD	mean	SD	mean	SD
alanine	9.5	0.33	23.1	0.79	38.6	1.98	37.6	0.81
glycine	9.4	0.16	67.9	2.13	9.6	0.38	115.0	4.52
valine	1.8	0.08	4.7	0.03	25.0	0.59	10.3	0.32
leucine	0.5	0.01	1.6	0.06	4.6	0.20	4.1	0.07
isoleucine	2.0	0.14	5.4	0.18	8.3	0.28	9.9	0.60
proline	2.2	0.09	5.2	0.15	9.9	0.20	5.4	0.25
threonine	16.2	0.32	11.6	0.48	22.6	0.90	2.3	0.12
serine	4.0	0.05	12.3	0.34	23.0	0.61	16.0	0.45
glutamic acid	6.3	0.43	23.0	0.34	49.6	1.77	5.7	0.12
asparagine	70.2	2.92	213.3	1.21	178.8	8.75	720.5	20.85
aspartic acid	259.0	13.02	214.9	2.70	387.9	21.55	448.4	18.58
methionine	0.2	0.01	2.2	0.09	0.1	0.01	1.0	0.01
hydroxyproline	5.7	0.24	116.6	8.88	55.4	1.11	55.1	3.92
phenylalanine	0.3	0.01	3.6	0.02	2.1	0.02	4.1	0.17
cysteine	25.0	0.64	7.5	0.13	55.5	1.09	6.0	0.04
glutamine	2.3	0.09	4.5	0.05	5.5	0.06	4.1	0.10
ornithine	1.1	0.03	3.4	0.04	1.1	0.08	1.6	0.06
lysine	5.2	0.16	8.9	0.09	5.1	0.19	6.1	0.20
histidine	2.4	0.21	5.2	0.14	0.3	0.02	28.2	0.71
tyrosine	0.3	0.01	0.2	0.01	1.4	0.01	2.3	0.17
tryptophan	0.6	0.05	1.6	0.02	0.1	0.01	1.5	0.12
Σ	424.1		736.8		884.6		1485.2	

[a] Values are expressed as the mean of three determinations; SD, standard deviation; Σ, sum of the determined free amino acids.

Since none of the amino acids are volatile enough for direct GC analysis, it is necessary to transform them into volatile derivatives. The chosen amino acids derivatization procedure with ethyl chloroformate is unique in rapidity, although one drawback is the inability to determine arginine (*15*−*19*). Not all reactive groups in the amino acids are altered by action of the reagent. The imino group of arginine remains untouched, which is the reason for the absorption of this amino acid

Table 5. Free Amino Acids Composition of Industrially Manufactured Quince Jams (μg/kg) (Quantification by Internal Standard Technique)[a]

	quince jams															
	E		F		G		H		I		J		K		L	
amino acids	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
alanine	13.3	0.47	9.2	0.29	68.1	1.05	14.4	0.37	61.2	0.65	110.3	1.75	8.3	0.16	10.1	0.61
glycine	80.2	3.38	77.5	2.10	65.3	1.92	112.7	4.39	5.5	0.06	9.6	0.20	29.1	1.53	45.2	2.57
valine	4.5	0.28	2.0	0.09	19.5	0.70	3.4	0.04	15.3	0.73	3.7	0.16	7.0	0.15	6.4	0.16
leucine	1.6	0.04	1.4	0.04	5.9	0.11	1.5	0.07	2.5	0.10	1.7	0.07	1.7	0.09	3.6	0.17
isoleucine	4.4	0.11	5.6	0.22	23.4	0.45	7.3	0.20	11.1	0.36	5.4	0.11	6.4	0.08	12.5	0.48
proline	2.6	0.06	2.9	0.10	6.2	0.12	2.4	0.09	3.5	0.14	4.6	0.18	5.3	0.22	3.1	0.02
threonine	13.1	0.33	9.9	0.33	7.7	0.23	16.4	0.50	23.2	0.57	31.0	1.07	15.0	0.77	9.9	0.39
serine	7.2	0.15	10.1	0.19	16.3	0.27	5.9	0.03	14.3	0.60	15.4	0.52	13.6	0.59	10.3	0.53
glutamic acid	4.9	0.27	26.5	0.34	25.0	1.13	29.2	0.57	76.3	3.75	61.3	1.78	38.3	1.52	16.0	0.79
asparagine	83.7	2.53	47.2	1.22	219.0	7.71	20.3	0.60	68.1	1.57	40.7	1.42	244.7	6.83	65.9	2.31
aspartic acid	262.1	8.06	153.7	2.81	51.0	1.13	198.9	5.59	331.1	13.07	197.9	3.65	413.9	17.09	33.7	0.94
methionine	0.1	0.01	0.1	0.01	0.4	0.02	0.4	0.01	1.5	0.01	0.4	0.02	0.1	0.01	0.3	0.01
hydroxyproline	5.2	0.11	11.9	0.80	41.5	0.65	11.5	0.19	325.5	11.71	11.9	0.16	5.8	0.30	27.9	0.74
phenylalanine	1.5	0.09	1.0	0.06	4.0	0.12	2.2	0.06	2.2	0.14	2.6	0.03	6.7	0.19	6.7	0.05
cysteine	9.3	0.60	8.1	0.27	0.4	0.01	9.6	0.22	119.1	4.14	38.9	1.65	21.8	0.77	7.6	0.68
glutamine	5.0	0.20	6.4	0.17	5.8	0.23	7.6	0.54	17.2	1.17	8.6	0.36	7.1	0.04	4.8	0.32
ornithine	0.7	0.01	1.0	0.05	4.8	0.30	0.6	0.02	0.6	0.03	1.3	0.03	0.5	0.01	3.7	0.33
lysine	6.5	0.26	7.5	0.16	14.9	0.35	3.7	0.07	3.5	0.15	8.2	0.16	8.3	0.25	8.9	0.38
histidine	5.6	0.43	7.7	0.04	10.7	0.61	1.4	0.06	1.5	0.02	4.0	0.09	5.8	0.06	27.0	0.61
tyrosine	0.6	0.01	1.0	0.06	1.9	0.10	0.3	0.01	3.1	0.14	0.7	0.01	0.5	0.01	1.1	0.06
tryptophan	0.6	0.01	0.1	0.01	1.3	0.05	1.6	0.07	0.2	0.01	0.2	0.01	2.5	0.04	4.6	0.24
Σ	512.6		390.6		593.0		451.0		1086.5		558.5		842.7		309.2	
	quince jams															
	M		N		O		P		Q		R		S		T	
amino acids	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
alanine	7.4	0.30	21.8	0.87	10.7	0.24	15.9	1.01	198.6	6.04	10.5	0.06	24.1	0.33	68.4	0.62
glycine	21.9	0.99	58.4	2.11	86.9	2.21	9.2	0.44	86.1	1.79	102.0	4.12	57.2	1.46	9.8	0.49
valine	5.4	0.12	6.5	0.33	7.5	0.20	7.2	0.29	6.3	0.24	7.3	0.21	5.0	0.02	6.9	0.23
leucine	1.8	0.06	1.5	0.06	2.5	0.07	3.4	0.10	0.8	0.04	1.6	0.04	1.4	0.01	5.1	0.06
isoleucine	5.2	0.13	6.2	0.21	9.4	0.18	7.7	0.17	80.1	0.42	7.9	0.19	5.9	0.27	9.3	0.04
proline	2.5	0.09	2.2	0.05	3.8	0.05	4.0	0.08	1.4	0.08	2.2	0.10	1.9	0.11	10.2	0.15
threonine	19.1	0.39	18.0	0.34	20.2	0.35	29.1	1.29	8.2	0.08	21.1	1.04	55.5	2.12	44.5	0.97
serine	7.0	0.09	11.9	0.24	10.8	0.73	36.2	1.56	8.9	0.48	6.3	0.17	5.1	0.29	29.3	1.21
glutamic acid	9.6	0.11	20.8	0.99	24.0	0.86	18.5	0.92	23.4	0.16	19.4	0.39	10.3	0.15	46.2	1.82
asparagine	82.4	3.26	142.5	1.07	174.1	1.47	95.0	3.52	125.3	4.88	80.8	0.61	92.3	5.30	293.7	10.16
aspartic acid	215.3	3.11	324.8	6.25	390.0	3.55	158.9	11.20	151.7	0.19	291.5	4.76	392.3	16.07	391.1	13.17
methionine	0.1	0.01	0.9	0.06	0.2	0.01	0.1	0.01	0.1	0.01	0.1	0.01	0.2	0.02	0.3	0.01
hydroxyproline	17.9	0.30	96.3	3.29	166.0	5.55	68.7	3.20	21.8	0.24	5.1	0.33	144.5	7.34	26.8	0.79
phenylalanine	2.5	0.12	3.4	0.10	2.1	0.02	1.0	0.03	1.3	0.03	1.7	0.02	0.3	0.02	4.2	0.10
cysteine	19.4	0.22	25.6	0.97	20.2	1.21	68.1	3.24	15.4	0.13	14.9	0.66	9.5	0.25	41.0	1.11
glutamine	5.6	0.06	0.3	0.02	16.0	0.94	16.0	0.60	13.2	0.55	10.9	0.72	1.0	0.02	21.3	0.26
ornithine	1.0	0.01	1.5	0.05	1.4	0.08	1.2	0.02	0.6	0.02	1.4	0.05	0.5	0.04	1.2	0.03
lysine	11.0	0.18	12.7	0.34	9.4	0.41	4.8	0.01	2.9	0.07	7.9	0.04	2.0	0.03	8.2	0.12
histidine	2.9	0.18	7.9	0.01	8.4	0.08	0.9	0.06	1.9	0.01	10.7	0.47	4.4	0.25	7.8	0.04
tyrosine	0.3	0.02	1.0	0.06	1.7	0.10	2.3	0.07	0.3	0.02	0.4	0.02	0.7	0.03	3.0	0.10
tryptophan	1.1	0.03	0.1	0.01	1.3	0.04	0.1	0.01	0.8	0.01	1.7	0.01	0.3	0.01	1.0	0.03
Σ	439.1		764.2		966.7		548.1		749.1		605.7		814.5		1029.3	

[a] Values are expressed as the mean of three determinations; SD, standard deviation; Σ, sum of the determined free amino acids.

derivative in the column (*15*−*19*). For the determination of arginine, an additional reaction step would be necessary or, alternatively, its conversion into ornithine by arginase before derivatization (*15*) would be required.

Because the free amino acid pattern is typical of a fruit and hence can be utilized for the analytical characterization of a fruit product (*1*), samples of quince fruit (pulp and peel) from seven different geographic origins from Portugal were analyzed. As expected, quince fruit is a food matrix with very low free amino acids content. The sum of the 21 free amino acids ranged from approximately 316 to 1357 μg/kg for quince pulps and from 512 to 1820 μg/kg for quince peels (**Tables 2** and **3**). As a general rule, the amount of free amino acids was higher in peels than in the corresponding pulps, with the exceptions of quinces from Vila Real and Pinhel. Nevertheless, the difference between the mean value of total free amino acids content from quince pulps (807.2 μg/kg; $n = 7$) and the mean value from quince peels (1009.9 μg/kg; $n = 7$) was not significant ($p > 0.05$). All peels, except the one from Amarante, were characterized by higher absolute and percent amounts of glycine than the correspondent pulps, although the difference between the mean value of the glycine content (and percent) from quince pulps (69.8 μg/kg; $n = 7$) and from quince peels (217.6 μg/kg; $n = 7$) was not significant ($p > 0.05$).

In what concerns the quince pulps, generally the three most abundant free amino acids were aspartic acid, hydroxyproline, and asparagine. Free amino acids such as methionine, ornithine, and tyrosine were always present in very low amounts (<1%).

As a general rule, the three most abundant compounds present in quince peels were glycine, aspartic acid, and asparagine. The content in leucine, methionine, ornithine, and tyrosine was always very low (<1%).

It seems that the free amino acids content was related to the quince fruit geographical origin. However, other factors, such as cultural practices and degree of maturation, may have caused the observed differences.

Because pear and apple also belong to the *Rosaceae* family, it is important to compare the results herein obtained with those published for pear and apple. Asparagine, aspartic acid, glutamic acid, and serine are the major free amino acids of apple (*1, 3, 4*) and account for roughly 80% of the total free amino acids content (*3, 4*). According to Belitz and Grosch (*1*), the main free amino acid of pear is proline, but considerable percentages of aspartic acid, asparagine, glutamic acid, serine, and alanine are also present. Gomis et al. (*3*) reported that secondary amino acids, such as proline, are present in small amounts in apple, which means that pear is distinctive for its relatively high levels of proline compared to apple (*20*). According to Lea (*20*) and van Gorsel et al. (*21*), asparagine and glutamic acid account for the majority of free amino acids in pear and apple juices, followed by aspartic acid (*20, 21*). Pear juice also contains considerable amounts of serine, proline, valine, isoleucine, cysteine, and histidine (*21*). In what concerns apple juice, it also presents considerable content in serine, alanine, isoleucine, and histidine (*20, 21*). van Gorsel et al. (*21*) did not found any traces of tyrosine in apple juice or methionine in pear juice.

It seems that quince has some similarities with apple and pear; asparagine and aspartic acid are usually two of the major free amino acids. Sometimes, as in apple and/or pear, glutamic acid, cysteine, and histidine are the most abundant free amino acids. A high content of glycine and/or a comparatively high amount of hydroxyproline seems to be characteristic of quince fruit. Proline is present in small levels in quince (≤2.4%), such as in apple.

A total of 20 quince jam samples (4 homemade and 16 industrially manufactured) were analyzed. For all homemade quince jams, the two most abundant free amino acids were aspartic acid and asparagine and the third was cysteine, hydroxyproline, or glycine (**Table 4**). Many free amino acids (leucine, isoleucine, methionine, phenylalanine, glutamine, ornithine, tyrosine, and tryptophan) were present in very low amounts (<1%).

As in quince pulps and peels, in the industrially manufactured quince jam samples, the three major free amino acids were generally aspartic acid, asparagine, and glycine or hydroxyproline (**Table 5**). According to Portuguese legislation (*22*), quince jam is the food product of the homogeneous and consistent mixture, obtained exclusively by boiling quince mesocarp with sugars. It is possible that samples that had glycine as one of the three major free amino acids (samples D−H, L, M, and R) were prepared with unpeeled quinces. In some cases, amino acids such as alanine, glutamic acid, and cysteine were also among the three major amino acids. Probably the high alanine percentages (>8%) (samples G, J, and Q) were false because of addition of pear. A high content of proline could also be related to pear addition, but all quince jams had low percentages of this free amino acid (≤1%). Proline, methionine, and tyrosine content was always inferior to 1%.

In **Table 6** are presented the mean values (and SD) of free amino acids obtained for pulps, peels, and jams. Considering the total free amino acid content of quince pulps and peels and the usual fruit content of quince jams (ranging from 40% to 50%), some samples (D, I, O, and T) had higher amounts than expected. This may be due to the natural variability of fruits and/or to a possible hydrolysis of proteins, peptides, or other compounds with amino acids in their constitution, which can occur during the thermal processing (in acid medium).

In conclusion, although the free amino acid profile is not as suitable as the phenolic one (*9, 13*) for the detection of false readings of quince jam with quince peel and/or pear and apple, it may be a useful tool for this purpose.

Table 6. Free Amino Acids Composition of Quince Pulps, Peels, and Jams (μg/kg) (Mean Values)[a]

	samples					
	pulps		peels		jams	
amino acids	mean	SD	mean	SD	mean	SD
alanine	32.7	23.58	31.3	19.59	38.1	46.71
glycine	69.8	66.98	217.6	133.97	52.9	37.78
valine	17.9	14.58	23.3	18.91	7.8	5.82
leucine	5.6	4.84	5.3	5.99	2.4	1.50
isoleucine	21.4	21.97	42.5	38.94	11.7	16.69
proline	9.4	6.07	11.0	7.29	4.1	2.44
threonine	13.1	11.41	15.5	16.36	19.7	12.70
serine	35.1	23.96	44.0	45.42	13.2	8.20
glutamic acid	64.2	50.11	84.3	73.48	26.7	19.06
asparagine	133.4	91.35	121.6	57.99	152.9	153.10
aspartic acid	189.9	133.12	198.3	115.08	263.4	120.72
methionine	2.4	1.72	1.8	1.29	0.4	0.56
hydroxyproline	102.1	95.12	87.3	73.84	61.1	78.83
phenylalanine	6.5	5.56	5.7	4.96	2.7	1.81
cysteine	33.0	22.21	31.5	13.48	26.1	28.01
glutamine	14.9	13.16	12.4	10.68	8.2	5.77
ornithine	2.1	2.69	2.7	2.86	1.5	1.16
lysine	15.6	10.94	19.2	15.55	7.3	3.29
histidine	27.6	39.48	41.2	29.67	7.2	7.64
tyrosine	1.0	1.12	1.6	1.94	1.2	0.93
tryptophan	9.4	7.89	11.8	18.86	1.1	1.08
Σ	807.2	363.59	1009.9	490.59	709.6	287.67

[a] SD, standard deviation; Σ, sum of the determined free amino acids.

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