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Quince (*Cydonia oblonga* Miller) Fruit Characterization Using Principal Component Analysis

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This paper presents a large amount of data on the composition of quince fruit with regard to phenolic compounds, organic acids, and free amino acids. Subsequently, principal component analysis (PCA) is carried out to characterize this fruit. The main purposes of this study were (i) the clarification of the interactions among three factors—quince fruit part, geographical origin of the fruits, and harvesting year—and the phenolic, organic acid, and free amino acid profiles; (ii) the classification of the possible differences; and (iii) the possible correlation among the contents of phenolics, organic acids, and free amino acids in quince fruit. With these aims, quince pulp and peel from nine geographical origins of Portugal, harvested in three consecutive years, for a total of 48 samples, were studied. PCA was performed to assess the relationship among the different components of quince fruit phenolics, organic acids, and free amino acids. Phenolics determination was the most interesting. The difference between pulp and peel phenolic profiles was more apparent during PCA. Two PCs accounted for 81.29% of the total variability, PC1 (74.14%) and PC2 (7.15%). PC1 described the difference between the contents of caffeoylquinic acids (3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids and 3,5-*O*-dicaffeoylquinic acid) and flavonoids (quercetin 3-galactoside, rutin, kaempferol glycoside, kaempferol 3-glucoside, kaempferol 3-rutinoside, quercetin glycosides acylated with *p*-coumaric acid, and kaempferol glycosides acylated with *p*-coumaric acid). PC2 related the content of 4-*O*-caffeoylquinic acid with the contents of 5-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids. PCA of phenolic compounds enables a clear distinction between the two parts of the fruit. The data presented herein may serve as a database for the detection of adulteration in quince derivatives.

KEYWORDS: ***Cydonia oblonga*** **Miller; quince fruit; pulp; peel; phenolic compounds; organic acids; free amino acids; principal component analysis**

INTRODUCTION

Quince is the fruit of a deciduous tree of the Rosaceae family, *Cydonia oblonga* Miller. Although quince fruit is not edible raw because of its hardness, bitterness, and astringency, it is very appreciated in Portugal for its jam, called "marmelada". According to Portuguese legislation (*1*), quince jam is the food product of a homogeneous and consistent mixture obtained exclusively by boiling quince mesocarp with sugars.

Before 1998, only a few chemical studies have been developed in this matrix. These works concerned mainly the volatile constituents of quince fruit (*2*−*7*) and the glucosides of procyanidin polymers (*8*).

For the past few years, quince fruit and its derivatives have been studied by our research group to examine their chemical constituents (*9*−*19*) and to evaluate their antioxidant potential (*20*). Among the various studied chemical parameters, the phenolic profile seemed to be the most useful in the discrimination of the different parts of quince fruit (pulp, peel, and seed) (*10*, *14*, *15*). This procedure also allowed the detection of adulterations in quince jams by the addition of quince peel (*10*).

As the published literature was based on results from only one year of quince harvest (2000), and considering the pos-

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Table 1. Phenolic Composition of Quince Pulps[a]

obser-	geographical		phenolic compound (%)												
vation	origin	year	3-CQA	SD	4-CQA	SD	5-CQA	SD	3,5-diCQA	SD	Q-3-Gal	SD	Q-3-Rut	SD	Σ (mg/kg)
1	Amarante	2000	24.15	0.342	4.46	0.046	59.28	0.763	8.03	0.063	nd		4.08	0.149	134.3
2		2001	28.87	0.992	17.74	0.676	50.11	1.249	2.46	0.009	0.24	0.012	0.57	0.020	167.1
3		2002	45.44	0.354	4.11	0.007	43.23	0.521	4.46	0.031	1.19	0.011	1.57	0.020	208.1
4	Baião	2000	16.84	0.436	2.90	0.259	69.11	0.692	5.95	0.277	nd		5.20	0.580	142.2
5		2001	21.12	0.349	1.73	0.070	71.55	0.947	2.51	0.124	nd		3.09	0.036	135.6
6		2002	22.15	0.381	2.20	0.025	69.81	1.115	4.35	0.032	nd		1.49	0.087	364.8
7	Bragança	2000	7.63	0.045	4.87	0.014	47.80	0.776	tr		nd		39.71	0.682	11.7
8		2001	32.74	0.648	2.53	0.068	62.79	0.273	1.94	0.041	nd		nd		162.4
9		2002	41.37	0.624	4.95	0.008	46.47	0.902	4.72	0.063	nd		2.49	0.087	160.1
10	Caminha	2001	49.10	0.547	7.32	0.103	43.58	0.298	tr		nd		nd		154.1
11	Covilhã	2000	22.28	0.707	3.38	0.007	69.30	0.434	2.37	0.062	nd		2.66	0.093	155.9
12		2001	35.84	0.014	4.08	0.183	54.32	0.926	3.51	0.087	nd		2.25	0.041	206.7
13		2002	29.27	0.591	5.15	0.261	60.76	0.433	3.35	0.117	nd		1.46	0.041	260.9
14	Custóias	2001	26.07	0.254	2.72	0.235	65.83	1.922	1.93	0.023	0.89	0.021	2.57	0.053	322.9
15		2002	32.41	0.816	9.72	0.236	54.34	0.029	2.63	0.040	0.30	0.001	0.59	0.006	518.6
16	Pinhel	2000	20.98	0.259	2.73	0.019	69.17	0.861	2.36	0.076	1.57	0.118	3.19	0.024	268.3
17		2001	37.33	0.069	2.95	0.032	57.25	0.279	2.47	0.016	nd		nd		343.8
18		2002	31.24	1.345	9.17	0.214	54.05	0.219	3.68	0.176	nd		1.85	0.036	365.0
19	Vila Real	2000	25.67	0.363	5.52	0.290	61.94	0.444	4.91	0.129	nd		1.96	0.116	88.3
20		2001	33.84	0.254	3.32	0.156	55.38	0.329	2.59	0.013	nd		4.87	0.604	136.5
21		2002	45.17	0.288	3.05	0.002	45.24	0.156	4.02	0.048	1.08	0.011	1.43	0.065	313.2
22	Viseu	2000	27.51	0.016	4.55	0.068	59.90	0.945	3.31	0.159	nd		4.73	0.522	109.7
23		2001	44.88	0.568	6.11	0.096	42.89	0.169	3.33	0.055	nd		2.78	0.163	212.8
24		2002	30.28	0.507	17.77	0.062	48.50	0.660	0.49	0.015	nd		2.97	0.028	434.1
mean			30.51		5.54		56.78		3.14		0.22		3.81		224.1
max			49.10		17.77		71.55		8.03		1.57		39.71		518.6
min			7.63		1.73		42.89		tr		nd		nd		11.7
SD			10.058		4.273		9.400		1.802		0.458		7.790		120.53

[a] SD, standard deviation of three determinations; nd, not detected; tr, traces; Σ, sum of the determined phenolics; 3-CQA, 3-*O*-caffeoylquinic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 3,5-CQA, 3,5-*O*-dicaffeoylquinic acid; Q-3-Gal, quercetin 3-galactoside; Q-3-Rut, rutin.

sibility of the influence of geographical origin and harvesting year on the chemical profile, the paper herein reports, for the first time, the phenolic, organic acid, and free amino acid composition of quince fruit harvested in 2001 and 2002. Principal component analysis (PCA) was applied to the results of the three years of quince harvest to determine the relationship among the different components of quince fruit phenolics, organic acids, and free amino acids. PCA and ANOVA were performed separately for each chemical parameter.

The main purposes of this study were (i) to clarify the interactions between the studied factors (quince fruit part, geographical origin of the fruits, and harvesting year) and the phenolic, organic acid, and free amino acid profiles; (ii) to classify the possible differences; and (iii) to verify if there is a correlation among the contents of phenolics, organic acids, and free amino acids in quince fruit. Finally, after the acquisition of these data, we indicate what is the most useful parameter with regard to the quality control of these food products.

MATERIALS AND METHODS

Samples. Healthy quince fruit samples were collected in different places in northern (Amarante, Baião, Vila Real, Bragança, Custóias, and Caminha) and central (Viseu, Pinhel, and Covilhã) Portugal, in 2000 (14 samples), 2001 (18 samples), and 2002 (16 samples). For each sample from each geographical origin, ∼1 kg of quince fruits was manually collected from around quince trees present in the quince orchard. All fruits were separated into pulp and peel. Each part of the fruit was cut in thin slices and freeze-dried. Lyophilization was carried out using a Labconco 4.5 apparatus (Kansas City, MO).

Standards. The standards were from Sigma (St. Louis, MO) and from Extrasynthése (Genay, France). Methanol and formic and hydrochloric acids were obtained from Merck (Darmstadt, Germany), and sulfuric acid was from Pronalab (Lisboa, Portugal). Ethyl chloroformate (ECF) was from Aldrich (Steinheim, Germany) and pyridine from Fluka (Neu-Ulm, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Solid-Phase Extraction (SPE) Columns. The Isolute C18 non-end-capped (NEC) SPE columns (50 μm particle size, 60 Å porosity; 10 g of sorbent mass/70 mL of reservoir volume) were purchased from International Sorbent Technology Ltd. (Mid Glamorgan, U.K.). The benzenesulfonic SCX Spe-ed SPE cartridges (200 mg; 3 mL) were obtained from Applied Separations (Allentown, PA).

Extraction and HPLC Analysis of Phenolic Compounds. The extraction of phenolics was achieved as previously reported (*9*, *10*, *15*, *19*). Briefly, each sample (∼1 g) was thoroughly mixed with water (pH 2 with HCl) until complete extraction of the phenolic compounds (negative reaction to 20% NaOH) and filtered. One percent methanol was added to the filtrate, which was then passed through an Isolute C18 (NEC) column, preconditioned with 60 mL of methanol and 140 mL of water (pH 2 with HCl). Sugars and other polar compounds were eluted with the aqueous solvent. The retained phenolic fraction was then eluted with methanol (.∼50 mL). The extract was concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (1 mL), and 20 μL was analyzed by HPLC.

Separation of the phenolics was achieved as reported previously (*9*, *10*, *15*−*20*), with an analytical HPLC unit (Gilson), using a Spherisorb 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 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min, and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min. Detection was achieved with a Gilson diode array detector. The compounds in each sample were identified by comparing

Table 2. Phenolic Composition of Quince Peels[a]

observation	geographical origin	year	phenolic compound (%)													
			3-CQA	SD	4-CQA	SD	5-CQA	SD	3,5-diCQA	SD	Q-3-Gal	SD	Q-3-Rut	SD	K-3-Gly	SD
25	Amarante	2000	10.72	0.257	1.53	0.066	26.65	0.083	3.50	0.159	2.39	0.055	39.56	4.354	2.18	0.017
26		2001	15.26	0.121	1.20	0.134	18.70	0.039	1.71	0.091	7.57	0.012	44.40	0.232	2.18	0.094
27		2002	13.40	0.295	1.22	0.039	17.86	0.550	1.55	0.131	3.88	0.339	47.29	1.797	2.06	0.100
28	Baião	2000	1.78	0.073	0.31	0.009	9.78	0.548	0.87	0.022	13.68	0.460	47.34	1.114	5.55	0.140
29		2001	4.22	0.105	0.96	0.009	16.56	0.316	1.01	0.088	10.13	0.159	45.66	0.044	3.68	0.018
30		2002	9.16	0.297	0.78	0.005	31.63	0.848	1.64	0.097	12.13	0.228	31.32	1.018	3.29	0.087
31	Bragança	2000	0.12	0.008	0.10	0.001	2.10	0.015	0.76	0.046	12.17	0.939	61.80	0.703	8.74	0.743
32		2001	7.35	0.007	2.53	0.046	26.74	0.327	tr		11.03	0.105	36.52	1.442	4.20	0.056
33	Caminha	2002	29.08	0.227	2.75	0.013	35.17	0.271	2.99	0.084	4.09	0.039	17.74	0.077	2.38	0.001
34		2001	19.88	0.460	2.70	0.062	27.84	0.450	1.02	0.017	4.77	0.158	32.68	0.133	1.88	0.142
35	Covilhã	2000	1.21	0.020	0.26	0.001	6.24	0.100	0.61	0.087	14.76	0.146	50.21	0.779	6.44	0.041
36		2001	12.27	0.010	2.43	0.002	24.20	0.377	1.89	0.053	7.36	0.050	36.31	0.129	3.42	0.028
37	Custóias	2002	12.57	0.269	8.15	0.254	38.10	0.528	2.27	0.065	6.36	0.048	22.91	0.824	2.18	0.064
38		2001	10.77	0.021	1.43	0.043	54.62	0.152	1.26	0.036	6.04	0.034	22.82	0.071	0.39	0.001
39		2002	23.99	0.200	2.23	0.046	43.07	0.543	1.71	0.008	3.62	0.071	14.58	0.303	2.58	0.045
40	Pinhel	2000	5.39	0.175	1.09	0.043	22.98	0.916	1.21	0.034	11.01	0.324	44.40	1.284	2.33	0.050
41		2001	10.49	0.117	1.40	0.078	23.45	0.151	1.51	0.130	11.69	0.188	36.43	0.883	3.06	0.104
42		2002	11.06	0.011	1.11	0.005	21.43	0.080	1.36	0.015	8.85	0.272	37.40	0.056	4.49	0.097
43	Vila Real	2000	12.08	0.300	1.91	0.026	26.85	0.821	2.69	0.127	8.45	0.290	39.07	1.605	1.37	0.023
44		2001	6.93	0.328	3.82	0.109	17.16	0.705	1.64	0.037	10.85	0.173	40.38	0.449	3.68	0.222
45		2002	18.57	0.388	1.21	0.001	24.26	0.442	2.02	0.091	8.55	0.103	33.09	0.759	2.42	0.024
46	Viseu	2000	5.33	0.081	0.83	0.028	12.67	0.133	1.29	0.060	tr		57.88	1.013	4.36	0.075
47		2001	21.41	0.512	1.65	0.078	21.16	0.815	2.44	0.105	8.24	0.303	31.81	1.248	2.73	0.050
48		2002	19.41	1.079	1.67	0.013	23.38	0.861	2.05	0.078	7.96	0.319	34.04	0.950	2.20	0.008
mean			11.77		1.80		23.86		1.63		8.15		37.74		3.24	
max			29.08		8.15		54.62		3.50		14.76		61.80		8.74	
min			0.12		0.10		2.10		tr		tr		14.58		0.39	
SD			7.426		1.613		11.478		0.787		3.743		11.437		1.773	

observation	geographical origin	year	phenolic compound (%)												
			K-3-Glu	SD[a]	K-3-Rut	SD[a]	Q-Gly-pC1	SD[a]	Q-Gly-pC2	SD[a]	K-Gly-pC1	SD[a]	K-Gly-pC2	SD[a]	Σ (mg/kg)
25	Amarante	2000	1.53	0.055	3.38	0.056	3.08	0.081	1.20	0.073	1.57	0.130	2.71	0.206	1093.8
26		2001	0.84	0.028	2.39	0.025	2.46	0.023	1.07	0.010	0.80	0.001	1.44	0.012	981.0
27		2002	1.65	0.026	3.26	0.072	3.32	0.059	1.13	0.003	1.17	0.006	2.20	0.012	1566.4
28	Baião	2000	5.04	0.143	7.61	0.100	1.96	0.065	1.34	0.051	tr		4.74	0.208	1843.0
29		2001	3.82	0.019	5.21	0.369	2.65	0.020	1.09	0.001	1.83	0.018	3.19	0.060	1417.3
30		2002	2.34	0.146	3.30	0.156	1.36	0.012	0.71	0.007	0.82	0.007	1.51	0.047	1306.2
31	Bragança	2000	3.05	0.039	7.24	0.210	1.59	0.017	0.54	0.023	0.87	0.088	0.92	0.107	278.8
32		2001	3.22	0.099	4.36	0.216	1.06	0.106	0.78	0.023	0.82	0.011	1.39	0.001	1173.9
33	Caminha	2002	1.84	0.011	2.39	0.002	0.45	0.012	0.18	0.003	0.32	0.003	0.62	0.011	812.3
34		2001	1.02	0.033	2.02	0.074	2.55	0.008	0.82	0.009	1.80	0.013	1.03	0.020	694.6
35	Covilhã	2000	4.88	0.074	6.55	0.055	2.34	0.004	1.73	0.033	1.65	0.025	3.14	0.037	935.2
36		2001	2.23	0.058	4.46	0.004	1.54	0.005	0.77	0.002	1.08	0.004	2.04	0.055	758.0
37	Custóias	2002	1.41	0.091	2.39	0.111	1.20	0.081	0.47	0.002	0.88	0.081	1.11	0.038	1165.9
38		2001	0.24	0.001	0.33	0.001	1.16	0.007	0.48	0.002	0.29	0.005	0.17	0.001	1284.9
39		2002	2.07	0.009	2.75	0.039	0.68	0.014	0.34	0.018	0.77	0.026	1.61	0.020	632.4
40	Pinhel	2000	2.22	0.038	4.05	0.069	1.85	0.027	0.85	0.037	0.74	0.009	1.87	0.011	1882.8
41		2001	2.81	0.075	3.45	0.142	2.19	0.082	0.92	0.026	0.88	0.072	1.73	0.137	1962.4
42		2002	3.90	0.193	5.82	0.284	1.12	0.037	0.55	0.003	1.04	0.016	1.86	0.063	1695.6
43	Vila Real	2000	0.91	0.024	2.29	0.090	1.58	0.046	0.81	0.009	0.54	0.002	1.45	0.004	571.3
44		2001	3.05	0.022	4.91	0.120	2.18	0.028	1.39	0.008	1.41	0.108	2.60	0.241	1382.8
45		2002	1.65	0.021	3.01	0.064	1.90	0.127	0.77	0.080	0.77	0.093	1.77	0.162	1118.5
46	Viseu	2000	3.50	0.043	7.50	0.108	1.81	0.017	0.93	0.020	1.34	0.080	2.56	0.001	1062.0
47		2001	1.80	0.074	3.23	0.088	2.01	0.072	0.91	0.040	tr		2.61	0.113	1105.7
48		2002	1.56	0.037	2.97	0.056	1.81	0.052	0.67	0.012	0.74	0.010	1.53	0.051	1517.0
mean			2.36		3.95		1.83		0.85		0.92		1.91		1176.7
max			5.04		7.61		3.32		1.73		1.83		4.74		1962.4
min			0.24		0.33		0.45		0.18		tr		0.17		278.8
SD			1.249		1.890		0.708		0.354		0.503		0.968		435.40

[a] SD, standard deviation of three determinations; tr, traces; Σ, sum of the determined phenolics; 3-CQA, 3-*O*-caffeoylquinic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 3,5-CQA, 3,5-*O*-dicaffeoylquinic acid; Q-3-Gal, quercetin 3-galactoside; Q-3-Rut, rutin; K-3-Gly, kaempferol 3-glycoside; K-3-Glu, kaempferol 3-glucoside; K-3-Rut, kaempferol 3-rutinoside; Q-Gly-pC1 and Q-Gly-pC2, quercetin glycosides acylated with *p*-coumaric acid; K-Gly-pC1 and K-Gly-pC2, kaempferol glycosides acylated with *p*-coumaric acid.

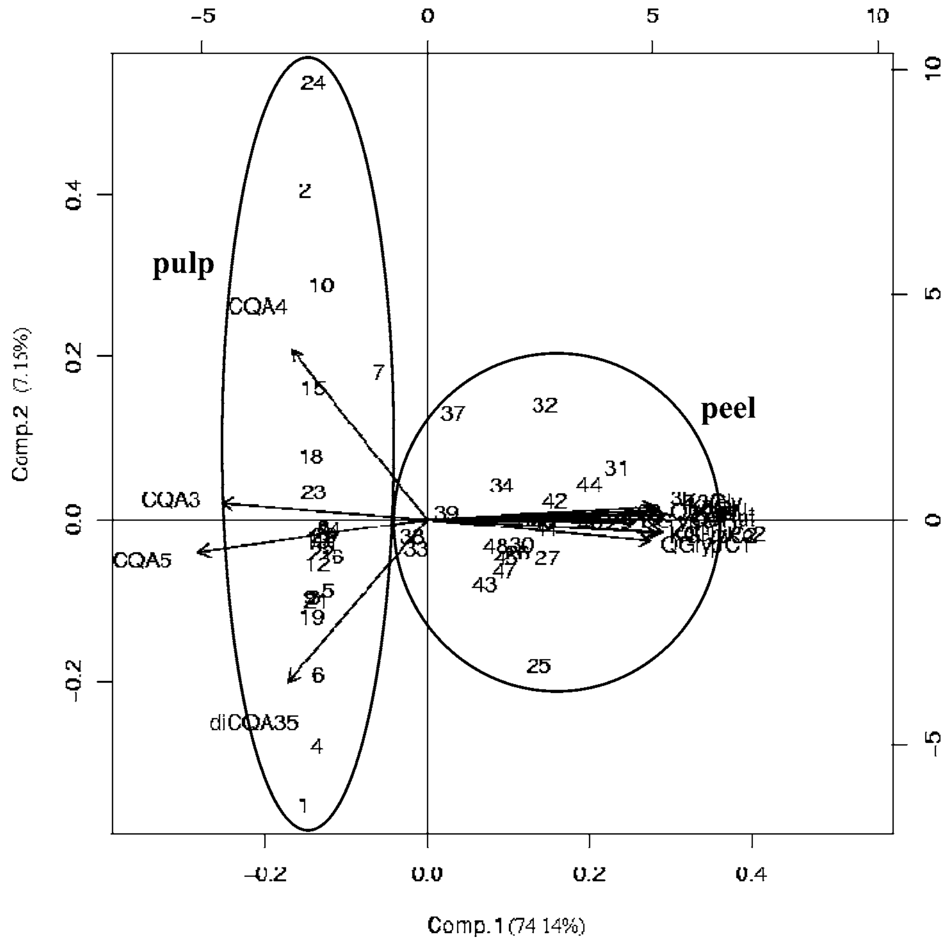

Figure 1. PCA of phenolic compounds in quince fruit, from 48 independent observations. CQA3, 3-*O*-caffeoylquinic acid; CQA4, 4-*O*-caffeoylquinic acid; CQA5, 5-*O*-caffeoylquinic acid; diCQA35, 3,5-*O*-dicaffeoylquinic acid; Q3Gal, quercetin 3-galactoside; Q3Rut, rutin; K3Gly, kaempferol 3-glycoside; K3Glu, kaempferol 3-glucoside; K3Rut, kaempferol 3-rutinoside; QGlypC1 and QGlypC2. quercetin glycosides acylated with *p*-coumaric acid; KGlypC1 and KGlypC2, kaempferol glycosides acylated with *p*-coumaric acid.

their retention times and UV−vis spectra in the 200−400 nm 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.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. 3- and 4-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids were quantified as 5-*O*-caffeoylquinic acid. Kaempferol glycoside and kaempferol glycosides acylated with *p*-coumaric acid were quantified as kaempferol 3-glucoside. Quercetin glycosides acylated with *p*-coumaric acid were quantified as quercetin 3-galactoside. The other compounds were quantified as themselves.

Extraction and HPLC Analysis of Organic Acids. The sample preparation was performed as reported by Silva et al. (*11*, *15*, *19*). Briefly, each sample (∼1 g) was thoroughly mixed with methanol (10 × 50 mL) (40 °C). The methanolic extract was filtered, concentrated to dryness under reduced pressure (40 °C), and redissolved in acid water (pH 2 with HCl) (∼50 mL). The aqueous solution was 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 extract was evaporated to dryness under reduced pressure (40 °C) and redissolved in sulfuric acid 0.01 N (5 mL), and 20 μL was analyzed by HPLC.

The separation was carried out as previously reported (*11*, *15*, *19*, *20*), with an analytical HPLC unit (Gilson), using a ion exclusion column Nucleogel Ion 300 OA (300 × 7.7 mm), in conjunction with a column heating device at 30 °C. Elution was carried out at a solvent flow rate of 0.1 mL/min, isocratically with 0.01 N sulfuric acid as the mobile phase. Detection was performed with an Gilson UV detector 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. The other acids were quantified as themselves.

Extraction and GC Analysis of Free Amino Acids. Extraction was conducted according to the method of Silva et al. (*12*, *13*, *15*, *19*). Briefly, each sample (∼1.5 g) 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 an 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 (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 a N_2 stream and kept below 0 °C until derivatization.

The derivatization of L-amino acids was carried out as reported previously (*12*, *13*, *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 ethyl chloroformate was added, and the solution was vortex-mixed (3−5 s). Five minutes later, 150 μL of dichloromethane and ∼0.01 g of NaCl were added, and the vial was thoroughly shaken for the 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.

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) (*12*, *13*, *15*, *19*). The injector and the detector were kept at 250 and

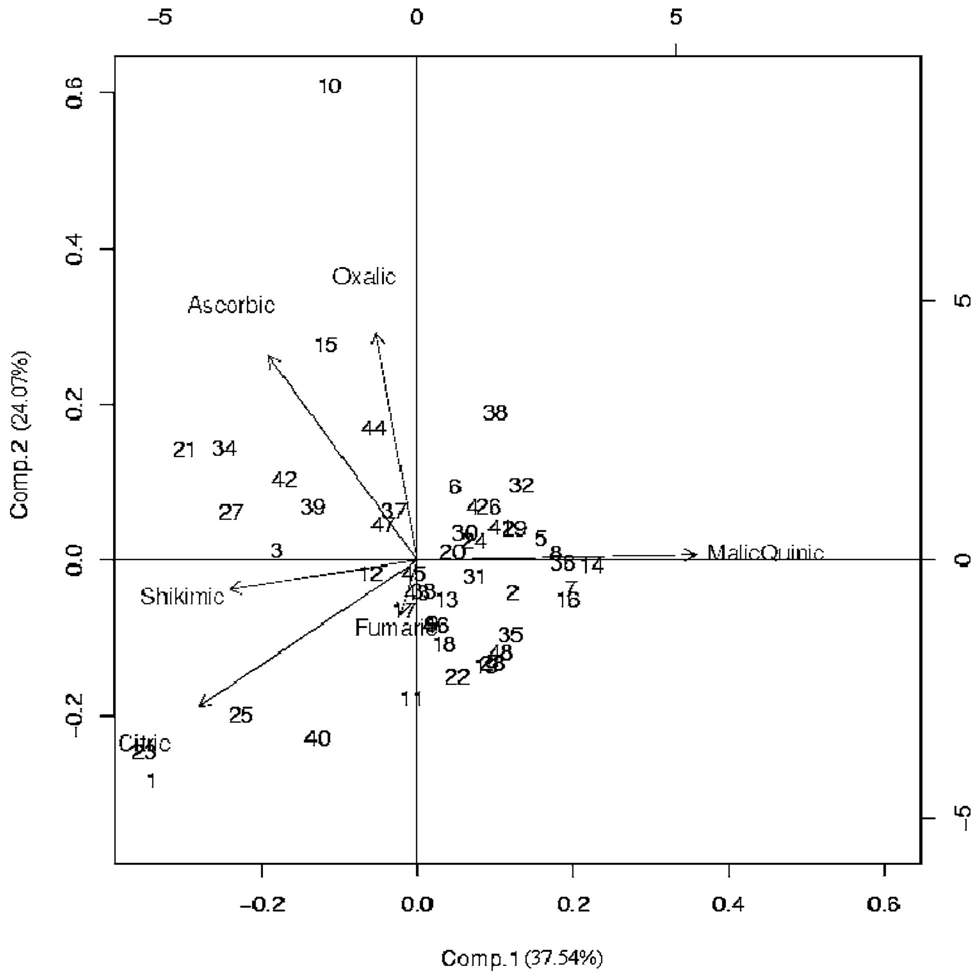

Figure 2. PCA of organic acids in quince fruit, from 48 independent observations.

280 °C, respectively. 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.

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

Statistical Analysis. *Experimental Design.* Quince pulp and peel were analyzed in terms of phenolics, organic acids, and free amino acids. The analysis comprised results from nine different locations from Portugal, throughout the harvesting years of 2000, 2001, and 2002. It was not possible to obtain quince fruit samples for every harvesting year. Therefore, the analysis was carried out with the partial factorial design without replication (*21*, *22*), totaling 48 samples. The factors that were evaluated were quince fruit part, geographical origin, and harvesting year. Factor combinations and responses are presented in **Tables 1**−**6**.

Software. All statistical analyses involving the experimental data were performed using R 1.9.0 for Linux (*23*).

Multifactor ANOVA. A multifactor ANOVA (without replication) was performed to evaluate the effects of the studied factors—quince fruit parts (pulp and peel), Portugal region (Amarante, Baião, Bragança, Caminha, Covilhã, Custóias, Pinhel, Vila Real, and Viseu), and harvesting year (2000−2002)—on phenolics, organic acids, and free amino acids.

The multifactor linear regression model was analyzed for residuals normality and skewness to assess the validity of the ANOVA analysis. Despicable factor effects were removed from the full linear model to improve the accuracy of the analysis. The ANOVA tables and factor probabilities and their combinations were obtained. The Tukey multicomparison test was used to perform pairwise comparisons among factor level means (*24*).

Correlations. Pearson correlation coefficients among phenolics, organic acids, and free amino acids were calculated to obtain the possible correlation among the different quince fruit constituents (*24*).

Principal Component Analysis. PCA was performed to assess the correspondences among the different components of quince fruit phenolics, organic acids, and free amino acids. PCA was performed separately for each chemical parameter studied (phenolic, organic acid, and free amino acid profiles) and also for the global data.

Principal components (PCs) were analyzed for their variance percentage and component coefficients, to determine their significance. The Gabriel plot (biplot), using optimal scaling, was performed to gain greater insight into the relationships between quince fruit components, to interpret the different groups of data (*25*).

RESULTS AND DISCUSSION

The analytical variation of the used methodologies is despicable, once these techniques were previously validated (*9*−*12*).

Phenolic Compounds. Generally, quince pulps presented a chemical profile composed of six identified phenolic compounds: 3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids, 3,5-*O*-dicaffeoylquinic acid, quercetin 3-galactoside, and rutin (**Table 1**), which is in accordance with previous studies (*10*, *19*, *20*). Usually, quince peels contained 13 phenolics: the 6 compounds present in pulps, plus kaempferol 3-glucoside, kaempferol 3-rutinoside, and 5 not totally identified compounds (1 kaempferol glycoside, 2 quercetin glycosides acylated with *p*-coumaric

Table 3. Organic Acid Composition of Quince Pulps[a]

obser-vation	geographical origin	year	organic acid (%) OA	SD	CA	SD	AA	SD	MA + QA	SD	SA	SD	FA	SD	Σ (mg/kg)
1	Amarante	2000	nd		8.42	0.141	0.80	0.011	90.46	0.127	0.32	0.004	tr		8162.3
2		2001	0.09	0.001	1.71	0.016	0.30	0.013	97.78	1.262	0.13	0.002	tr		10536.6
3		2002	0.12	0.001	3.88	0.038	2.14	0.089	93.53	0.406	0.33	0.001	tr		7416.1
4	Baião	2000	0.04	0.001	tr		2.29	0.080	97.45	1.968	0.22	0.002	0.01	0.001	6901.4
5		2001	0.08	0.001	0.35	0.010	0.93	0.005	98.50	1.353	0.14	0.002	nd		10670.0
6		2002	0.15	0.004	0.48	0.034	1.76	0.001	97.35	1.097	0.26	0.001	0.01	0.001	5337.1
7	Bragança	2000	nd		0.11	0.003	0.91	0.042	98.88	1.044	0.09	0.001	0.01	0.001	12786.4
8		2001	0.06	0.002	0.34	0.003	0.79	0.010	98.69	0.613	0.12	0.001	nd		17393.9
9		2002	0.02	0.001	1.67	0.052	0.73	0.017	97.20	0.389	0.38	0.007	nd		5860.7
10	Caminha	2001	0.67	0.009	0.43	0.011	3.91	0.028	94.77	0.245	0.22	0.001	nd		4794.1
11	Covilhã	2000	0.04	0.001	4.00	0.091	0.11	0.014	95.68	2.225	0.17	0.002	0.01	0.001	13962.1
12		2001	0.27	0.001	3.91	0.123	0.46	0.001	95.15	1.843	0.19	0.001	0.02	0.001	7397.0
13		2002	0.05	0.002	1.42	0.022	0.77	0.004	97.41	1.441	0.35	0.011	nd		6027.9
14	Custóias	2001	0.10	0.003	0.26	0.013	0.07	0.003	99.46	3.896	0.11	0.002	nd		8219.0
15		2002	0.22	0.013	0.95	0.047	3.88	0.087	94.65	0.096	0.30	0.005	nd		3497.0
16	Pinhel	2000	0.05	0.003	0.39	0.006	0.32	0.013	99.10	0.711	0.14	0.001	0.01	0.001	14185.8
17		2001	0.12	0.003	3.32	0.105	0.49	0.018	95.85	2.257	0.23	0.001	nd		6924.9
18		2002	nd		1.65	0.008	0.53	0.018	97.44	0.157	0.38	0.001	nd		3293.0
19	Vila Real	2000	tr		2.13	0.018	0.34	0.008	97.35	0.573	0.17	0.001	0.01	0.001	11284.4
20		2001	0.12	0.004	1.42	0.034	0.88	0.009	97.30	5.666	0.29	0.005	nd		6532.8
21		2002	0.21	0.001	4.39	0.015	3.72	0.064	91.40	0.708	0.28	0.001	0.01	0.001	4346.5
22	Viseu	2000	nd		2.62	0.024	0.39	0.008	96.80	0.665	0.19	0.001	0.01	0.001	9690.1
23		2001	0.06	0.002	6.37	0.155	0.56	0.052	92.31	1.325	0.68	0.001	0.01	0.001	2295.8
24		2002	0.04	0.002	0.45	0.003	1.53	0.119	97.69	0.692	0.28	0.001	nd		8367.2
mean			0.10		2.11		1.19		96.34		0.25		0.00		8161.8
max			0.67		8.42		3.91		99.46		0.68		0.02		17393.9
min			nd		tr		0.07		90.46		0.09		nd		2295.8
SD			0.141		2.157		1.174		2.431		0.127		0.006		3794.32

[a] SD, standard deviation of three determinations; nd, not detected; tr, traces; Σ, sum of the determined organic acids; OA, oxalic acid; CA, citric acid; AA, ascorbic acid; MA, malic acid; QA, quinic acid; SA, shikimic acid; FA, fumaric acid.

Table 4. Organic Acid Composition of Quince Peels[a]

obser-vation	geographical origin	year	organic acid (%) OA	SD	CA	SD	AA	SD	MA + QA	SD	SA	SD	FA	SD	Σ (mg/kg)
25	Amarante	2000	nd		4.65	0.134	1.00	0.018	93.80	0.023	0.55	0.002	0.01	0.001	4271.0
26		2001	0.12	0.003	0.73	0.020	1.36	0.129	97.60	1.182	0.19	0.001	tr		8987.8
27		2002	0.16	0.003	4.07	0.192	2.62	0.135	92.78	5.272	0.37	0.001	tr		7228.6
28	Baião	2000	tr		1.99	0.029	0.32	0.026	97.51	1.976	0.17	0.003	0.01	0.001	13511.1
29		2001	0.14	0.001	0.87	0.037	0.74	0.050	98.08	4.163	0.17	0.001	nd		12185.6
30		2002	nd		0.54	0.048	2.11	0.108	97.14	0.368	0.22	0.001	nd		4676.7
31	Bragança	2000	tr		0.62	0.042	1.60	0.027	97.53	4.361	0.24	0.002	0.01	0.001	7757.9
32		2001	0.18	0.002	0.48	0.024	1.00	0.024	98.19	0.322	0.15	0.001	nd		12583.4
33		2002	0.10	0.001	1.27	0.015	0.48	0.039	97.66	1.280	0.49	0.001	nd		5147.5
34	Caminha	2001	nd		1.75	0.143	4.76	0.258	93.03	2.892	0.46	0.027	nd		3859.7
35	Covilhã	2000	tr		1.00	0.007	0.53	0.026	98.23	0.009	0.23	0.005	0.01	0.001	13974.6
36		2001	0.13	0.001	0.55	0.016	tr		99.15	2.659	0.17	0.001	nd		13413.7
37		2002	0.21	0.002	1.94	0.026	1.07	0.104	96.42	1.725	0.36	0.003	nd		7001.1
38	Custóias	2001	0.27	0.003	0.37	0.013	1.45	0.018	97.74	3.178	0.16	0.007	nd		15511.9
39		2002	nd		1.55	0.001	3.37	0.124	94.68	0.127	0.40	0.001	nd		4125.5
40	Pinhel	2000	nd		5.87	0.738	0.57	0.012	93.40	3.248	0.15	0.002	0.01	0.001	13496.8
41		2001	0.11	0.001	0.36	0.001	0.98	0.099	98.28	4.379	0.27	0.003	nd		10414.5
42		2002	0.21	0.001	2.67	0.043	2.45	0.060	94.27	0.664	0.40	0.002	0.01	0.001	2533.7
43	Vila Real	2000	0.13	0.006	1.01	0.066	1.64	0.022	96.87	3.779	0.28	0.011	0.06	0.001	9160.4
44		2001	0.17	0.004	0.76	0.044	2.94	0.013	95.81	1.635	0.31	0.003	0.01	0.001	8203.4
45		2002	nd		1.64	0.092	1.85	0.034	96.23	0.105	0.27	0.003	nd		8276.0
46	Viseu	2000	nd		1.89	0.052	1.18	0.016	96.67	1.630	0.25	0.001	0.01	0.001	10769.3
47		2001	0.10	0.001	0.34	0.005	1.59	0.040	97.38	0.839	0.60	0.006	nd		4252.0
48		2002	nd		0.95	0.057	tr		98.68	1.815	0.37	0.013	nd		8182.0
mean			0.08		1.58		1.48		96.55		0.30		0.01		8730.2
max			0.27		5.87		4.76		99.15		0.60		0.06		15511.9
min			nd		0.34		tr		92.78		0.15		nd		2533.7
SD			0.088		1.437		1.128		1.893		0.131		0.012		3813.83

[a] SD, standard deviation of three determinations; nd, not detected; tr, traces; Σ, sum of the determined organic acids; OA, oxalic acid; CA, citric acid; AA, ascorbic acid; MA, malic acid; QA, quinic acid; SA, shikimic acid; FA, fumaric acid.

Table 5. Free Amino Acid Composition of Quince Pulps[a]

observation	geographical origin	year	Ala	SD	Gly	SD	Val	SD	Leu	SD	Ile	SD	Pro	SD	Thr	SD	Ser	SD	Glu	SD	Asn	SD	Asp	SD[a]
			amino acid (%)																					
1	Amarante	2000	2.45	0.196	12.88	0.540	2.61	0.116	0.91	0.030	2.61	0.050	0.95	0.040	0.50	0.042	1.86	0.145	7.93	0.491	17.23	0.517	15.06	0.989
2		2001	1.94	0.086	7.92	0.299	0.29	0.009	0.16	0.009	0.42	0.024	0.22	0.009	3.33	0.058	1.44	0.047	20.67	0.471	15.50	0.547	21.97	1.176
3		2002	3.38	0.079	0.29	0.011	0.85	0.033	0.13	0.004	2.46	0.063	0.42	0.018	2.09	0.085	7.03	0.075	21.84	0.992	4.75	0.151	28.84	1.024
4	Baião	2000	5.96	0.196	17.03	0.540	4.58	0.116	1.51	0.103	6.72	0.285	1.50	0.057	3.60	0.067	7.46	0.622	8.11	0.123	12.50	0.341	15.67	0.548
5		2001	1.00	0.011	46.69	0.747	0.22	0.005	0.08	0.002	1.80	0.024	0.14	0.007	1.44	0.036	0.83	0.065	10.62	0.257	9.72	0.461	13.79	0.304
6		2002	3.77	0.140	16.64	0.538	0.83	0.025	0.18	0.003	1.23	0.022	0.23	0.010	1.13	0.050	1.12	0.027	17.98	0.207	5.92	0.171	22.58	0.075
7	Bragança	2000	1.60	0.048	13.70	0.539	1.80	0.058	0.47	0.030	1.21	0.058	1.06	0.036	0.95	0.050	1.82	0.021	4.46	0.046	17.88	0.555	23.01	0.216
8		2001	1.24	0.037	32.61	1.545	0.96	0.039	0.08	0.002	1.96	0.039	0.15	0.007	1.42	0.005	1.93	0.049	10.56	0.260	18.73	0.707	9.06	0.253
9		2002	1.75	0.034	8.06	0.252	1.02	0.020	0.14	0.004	0.69	0.016	0.21	0.003	0.06	0.003	1.79	0.054	10.76	0.182	40.04	0.245	11.34	0.107
10	Caminha	2001	0.95	0.013	0.15	0.006	0.27	0.013	0.04	0.001	0.28	0.017	0.11	0.005	0.27	0.004	0.52	0.008	2.82	0.098	55.72	1.031	20.03	0.330
11	Covilhã	2000	2.44	0.094	0.83	0.036	0.78	0.028	0.35	0.023	2.01	0.125	0.58	0.028	2.53	0.142	8.91	0.119	9.45	0.415	9.44	0.063	32.25	1.753
12		2001	1.48	0.053	0.47	0.012	0.48	0.014	0.09	0.002	0.61	0.020	0.46	0.025	5.66	0.260	2.18	0.051	5.42	0.226	50.32	1.814	20.37	0.029
13		2002	1.47	0.036	3.93	0.047	0.71	0.018	0.12	0.002	0.31	0.005	0.18	0.003	0.33	0.013	0.49	0.007	9.77	0.050	45.70	0.061	15.86	0.337
14	Custóias	2001	0.61	0.009	3.86	0.092	0.84	0.009	0.09	0.002	0.38	0.006	0.23	0.003	2.66	0.039	1.33	0.070	7.68	0.230	27.58	0.353	22.01	0.197
15		2002	1.68	0.002	2.95	0.074	0.28	0.001	0.04	0.001	0.12	0.003	0.22	0.006	0.09	0.003	0.51	0.018	11.59	0.414	30.55	0.492	18.43	0.150
16	Pinhel	2000	3.59	0.103	3.47	0.211	1.55	0.080	0.49	0.008	1.56	0.042	0.65	0.033	0.76	0.023	2.44	0.121	12.29	0.859	8.95	0.262	33.97	1.139
17		2001	2.04	0.002	44.98	1.648	1.31	0.060	0.27	0.007	2.14	0.095	0.14	0.004	1.16	0.046	0.92	0.074	12.97	0.585	8.28	0.151	15.05	0.905
18		2002	3.32	0.046	4.53	0.173	2.70	0.116	0.41	0.008	0.98	0.033	0.44	0.013	0.84	0.031	4.99	0.201	20.57	0.510	28.63	0.945	13.42	0.355
19	Vila Real	2000	7.93	0.301	5.22	0.169	1.73	0.077	0.49	0.020	1.84	0.008	2.43	0.061	1.16	0.048	5.11	0.293	5.59	0.131	39.78	0.821	11.01	0.412
20		2001	2.17	0.023	17.86	0.433	0.71	0.007	0.13	0.007	0.96	0.032	0.31	0.010	0.39	0.038	1.79	0.097	10.85	0.176	31.99	1.107	14.89	0.464
21		2002	1.46	0.067	2.08	0.070	0.43	0.013	0.10	0.002	0.39	0.013	0.34	0.005	0.57	0.010	3.72	0.020	20.83	0.462	22.40	0.811	17.71	0.767
22	Viseu	2000	3.97	0.143	2.03	0.093	1.82	0.078	0.40	0.015	1.93	0.057	1.05	0.043	2.30	0.015	4.57	0.068	3.62	0.219	12.76	0.670	35.21	0.143
23		2001	1.38	0.164	1.69	0.065	0.49	0.043	0.19	0.010	0.24	0.007	0.25	0.009	2.42	0.045	0.70	0.010	12.79	0.276	28.30	1.341	26.31	0.839
24		2002	1.43	0.040	10.88	0.231	0.47	0.013	0.08	0.001	0.59	0.021	0.24	0.004	0.12	0.006	2.75	0.045	20.75	0.330	14.84	0.182	17.73	0.374
mean			2.46		10.86		1.16		0.29		1.39		0.52		1.49		2.76		11.66		23.23		19.82	
max			7.93		46.69		4.58		1.51		6.72		2.43		5.66		8.91		21.84		55.72		35.21	
min			0.61		0.15		0.22		0.04		0.12		0.11		0.06		0.49		2.82		4.75		9.06	
SD			1.693		13.238		1.012		0.332		1.374		0.543		1.366		2.383		5.895		14.651		7.219	

observation	geographical origin	year	Met	SD	Hyp	SD	Phe	SD	Cys	SD	Gln	SD	Orn	SD	Lys	SD	His	SD	Tyr	SD	Trp	SD	Σ (μg/kg)
			amino acid (%)																				
1	Amarante	2000	0.07	0.005	3.17	0.253	1.53	0.017	1.10	0.042	0.55	0.040	0.23	0.006	2.37	0.063	21.96	1.371	0.21	0.011	3.83	0.163	526.2
2		2001	0.09	0.004	18.90	0.444	0.76	0.026	0.54	0.021	1.81	0.060	0.11	0.008	0.41	0.019	3.15	0.184	0.04	0.004	0.34	0.011	1575.5
3		2002	0.24	0.009	7.95	0.278	0.26	0.022	9.77	0.141	2.66	0.068	0.45	0.004	1.68	0.037	4.18	0.151	0.27	0.008	0.45	0.004	480.4
4	Baião	2000	0.13	0.002	2.38	0.166	1.27	0.005	2.65	0.131	1.95	0.038	0.78	0.092	3.64	0.197	1.49	0.021	0.33	0.010	0.76	0.023	1044.6
5		2001	0.06	0.001	6.58	0.162	0.02	0.001	1.91	0.036	0.68	0.008	0.13	0.001	1.63	0.037	2.61	0.119	0.02	0.001	0.05	0.001	1424.9
6		2002	0.07	0.001	4.97	0.065	0.37	0.014	2.13	0.047	0.91	0.039	0.37	0.011	2.28	0.098	16.43	0.745	0.29	0.012	0.57	0.018	1355.7
7	Bragança	2000	0.17	0.010	19.49	0.393	0.28	0.016	2.27	0.087	3.31	0.219	0.15	0.011	2.03	0.081	2.51	0.048	0.04	0.001	1.77	0.085	1055.4
8		2001	0.15	0.013	16.66	0.844	0.03	0.001	1.83	0.028	0.50	0.007	0.11	0.005	0.57	0.012	1.19	0.060	0.16	0.009	0.11	0.001	1236.7
9		2002	0.34	0.016	9.58	0.470	0.34	0.008	0.66	0.008	0.12	0.002	0.09	0.002	0.68	0.006	10.84	0.093	0.08	0.001	1.42	0.015	1714.5
10	Caminha	2001	0.13	0.001	15.19	0.663	0.05	0.002	1.12	0.022	0.46	0.034	0.02	0.001	0.79	0.034	0.96	0.038	0.01	0.001	0.12	0.008	1587.3
11	Covilhã	2000	0.81	0.031	12.97	0.492	0.21	0.003	11.91	0.672	1.16	0.025	0.14	0.007	1.67	0.056	1.37	0.074	0.07	0.002	0.10	0.002	579.6
12		2001	0.39	0.013	2.06	0.051	0.04	0.001	4.24	0.158	1.86	0.031	0.04	0.001	0.66	0.007	3.14	0.146	0.01	0.001	0.04	0.001	390.8
13		2002	0.14	0.007	4.60	0.131	0.15	0.004	0.45	0.007	1.13	0.021	0.18	0.005	0.37	0.003	12.32	0.090	0.07	0.002	1.73	0.043	3113.9
14	Custóias	2001	0.02	0.002	30.26	1.597	0.09	0.001	0.67	0.031	0.62	0.029	0.01	0.001	0.21	0.007	0.64	0.024	0.07	0.002	0.14	0.001	3054.1
15		2002	0.07	0.001	11.88	0.327	0.20	0.003	0.16	0.008	0.77	0.014	0.18	0.004	1.03	0.014	18.03	0.670	0.04	0.001	1.18	0.033	2259.0
16	Pinhel	2000	0.35	0.016	19.50	0.376	1.05	0.038	4.15	0.223	2.12	0.022	0.10	0.002	0.90	0.053	1.11	0.095	0.09	0.003	0.92	0.029	1356.6
17		2001	0.01	0.001	4.70	0.194	0.10	0.005	1.69	0.105	0.71	0.056	0.16	0.011	0.43	0.011	2.93	0.161	0.02	0.002	nd		758.8
18		2002	0.60	0.007	5.93	0.252	0.34	0.010	2.71	0.068	1.01	0.008	0.02	0.001	1.04	0.032	6.67	0.280	0.13	0.001	0.72	0.026	946.9
19	Vila Real	2000	0.36	0.003	9.64	0.525	0.64	0.008	4.13	0.119	0.31	0.023	0.08	0.003	1.21	0.031	0.75	0.031	0.05	0.001	0.55	0.021	771.9
20		2001	0.05	0.002	9.53	0.371	0.38	0.007	0.74	0.016	0.32	0.014	0.16	0.003	1.24	0.024	5.22	0.215	0.07	0.005	0.24	0.003	707.1
21		2002	0.03	0.001	18.25	0.175	0.59	0.008	3.36	0.093	0.62	0.013	0.01	0.001	0.68	0.011	5.32	0.087	0.09	0.002	1.03	0.051	1918.3
22	Viseu	2000	0.34	0.024	16.85	0.834	0.19	0.005	5.24	0.268	2.60	0.052	0.42	0.003	1.99	0.082	2.05	0.043	0.06	0.003	0.59	0.026	315.9
23		2001	0.37	0.003	15.36	0.919	0.06	0.001	5.28	0.181	1.74	0.004	0.02	0.002	0.62	0.022	1.23	0.095	0.10	0.005	0.46	0.044	668.1
24		2002	0.09	0.001	12.98	0.319	0.80	0.005	0.73	0.025	0.56	0.022	0.13	0.004	0.64	0.028	12.77	0.432	0.08	0.002	1.31	0.035	1735.8
mean			0.21		11.64		0.41		2.89		1.19		0.17		1.20		5.79		0.10		0.77		1274.1
max			0.81		30.26		1.53		11.91		3.31		0.78		3.64		21.96		0.33		3.83		3113.9
min			0.01		2.06		0.02		0.16		0.12		0.01		0.21		0.64		0.01		nd		315.9
SD			0.199		6.994		0.412		2.905		0.863		0.177		0.821		6.184		0.089		0.844		762.03

[a] SD, standard deviation of three determinations; Σ, sum of the determined free amino acids; Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Ile, isoleucine; Pro, proline; Thr, threonine; Ser, serine; Glu, glutamic acid; Asn, asparagine; Asp, aspartic acid; nd, not detected; Σ, sum of the determined free amino acids; Met, methionine; Hyp, hydroxyproline; Phe, phenylalanine; Cys, cysteine; Gln, glutamine; Orn, ornithine; Lys, lysine; His, histidine; Tyr, tyrosine; Trp, tryptophan.

Table 6. Free Amino Acid Composition of Quince Peels[a]

obser-vation	geographical origin	year	Ala	SD	Gly	SD	Val	SD	Leu	SD	Ile	SD	Pro	SD	Thr	SD	Ser	SD	Glu	SD	Asn	SD	Asp	SD
			amino acid (%)																					
25	Amarante	2000	2.40	0.082	2.20	0.055	2.21	0.072	0.81	0.014	2.49	0.114	0.94	0.041	1.18	0.047	2.39	0.228	8.38	0.293	22.56	2.022	12.56	0.418
26		2001	2.06	0.099	2.69	0.056	0.08	0.002	0.11	0.002	0.47	0.010	0.37	0.006	2.46	0.048	1.03	0.041	19.27	0.947	36.22	0.492	15.57	0.320
27		2002	1.62	0.033	0.46	0.020	0.22	0.035	0.13	0.003	0.91	0.048	0.62	0.018	2.07	0.070	10.18	0.058	47.03	1.346	1.14	0.075	9.42	0.231
28	Baião	2000	3.85	0.073	19.51	0.347	3.46	0.067	1.01	0.015	6.89	0.121	1.37	0.024	2.85	0.037	8.00	0.115	12.73	0.284	6.19	0.118	13.89	0.336
29		2001	2.48	0.086	0.77	0.028	0.59	0.047	0.23	0.004	0.88	0.032	0.66	0.024	4.82	0.092	4.42	0.073	39.89	1.825	7.00	0.142	18.65	0.500
30		2002	3.48	0.150	23.02	0.863	0.12	0.003	0.18	0.006	2.11	0.092	0.26	0.011	2.17	0.096	1.61	0.090	26.77	0.814	3.39	0.163	19.77	0.961
31	Bragança	2000	1.48	0.079	23.11	1.367	1.78	0.061	0.37	0.014	3.62	0.113	0.91	0.020	0.85	0.057	1.93	0.129	7.52	0.321	13.53	0.278	20.96	1.043
32		2001	1.60	0.085	32.52	1.592	0.99	0.040	0.11	0.006	1.54	0.076	0.24	0.008	0.73	0.071	2.47	0.080	13.21	0.508	18.46	0.796	12.32	0.372
33		2002	3.92	0.106	16.91	0.686	0.96	0.036	0.16	0.003	1.51	0.055	0.39	0.012	1.35	0.069	1.36	0.005	12.45	0.352	19.10	0.362	23.28	2.471
34	Caminha	2001	1.52	0.028	0.73	0.015	0.08	0.001	0.11	0.002	0.12	0.007	0.33	0.003	1.70	0.039	0.44	0.010	14.43	0.445	33.78	1.541	28.69	0.652
35	Covilhã	2000	1.54	0.043	44.98	2.660	1.77	0.062	0.21	0.009	5.02	0.111	0.49	0.009	1.28	0.072	3.02	0.183	3.20	0.098	5.77	0.091	14.83	0.807
36		2001	1.11	0.067	5.46	0.277	0.48	0.016	0.10	0.003	0.56	0.034	0.37	0.007	1.42	0.063	0.65	0.003	10.83	0.360	36.84	0.795	10.43	0.070
37		2002	0.95	0.018	7.63	0.158	0.82	0.020	0.11	0.003	0.61	0.013	0.20	0.003	0.19	0.008	2.92	0.053	14.01	0.698	24.52	0.802	14.53	0.236
38	Custóias	2001	1.99	0.080	0.26	0.007	1.88	0.100	0.20	0.005	0.49	0.023	2.35	0.125	4.09	0.110	1.58	0.049	15.97	0.835	31.75	0.798	18.69	0.717
39		2002	1.64	0.022	5.60	0.178	0.57	0.016	0.06	0.002	0.26	0.007	0.35	0.008	0.21	0.007	3.75	0.110	15.08	0.439	24.56	0.474	17.48	0.378
40	Pinhel	2000	3.56	0.132	16.96	0.923	1.83	0.078	0.34	0.005	2.17	0.076	0.70	0.019	0.92	0.035	4.37	0.060	11.09	0.363	8.33	0.223	37.72	0.235
41		2001	0.91	0.029	40.05	1.580	0.96	0.033	0.17	0.009	1.81	0.038	0.10	0.004	0.90	0.029	0.98	0.058	20.70	0.673	9.58	0.243	13.42	0.503
42		2002	1.27	0.020	13.14	0.491	1.03	0.017	0.18	0.004	1.11	0.042	0.22	0.006	0.26	0.002	3.13	0.003	24.05	0.397	16.46	0.467	10.21	0.169
43	Vila Real	2000	7.14	0.079	19.41	1.452	1.26	0.053	0.26	0.006	4.37	0.124	1.85	0.083	2.83	0.121	5.50	0.302	7.28	0.032	19.92	0.713	11.99	0.488
44		2001	2.14	0.006	16.69	0.732	0.82	0.024	0.14	0.007	0.94	0.022	0.42	0.012	1.05	0.056	2.03	0.040	23.47	0.883	17.63	0.135	14.78	0.045
45		2002	1.28	0.056	13.13	0.594	0.59	0.001	0.12	0.007	0.75	0.021	0.39	0.012	0.40	0.011	2.29	0.057	24.21	0.795	13.40	0.455	17.49	0.799
46	Viseu	2000	3.28	0.103	23.48	0.519	2.40	0.062	0.22	0.011	2.44	0.059	1.31	0.036	0.47	0.019	3.03	0.128	3.42	0.082	17.45	0.621	23.85	1.038
47		2001	0.96	0.037	0.73	0.024	0.69	0.031	0.11	0.003	0.30	0.011	0.25	0.009	1.66	0.156	0.99	0.013	14.57	0.503	35.55	1.034	19.52	0.330
48		2002	3.08	0.061	1.02	0.036	0.86	0.035	0.17	0.006	0.84	0.042	0.55	0.007	1.51	0.045	3.27	0.123	42.77	0.933	4.21	0.013	18.38	0.956
mean			2.30		13.77		1.10		0.23		1.76		0.65		1.56		2.97		18.01		17.81		17.43	
max			7.14		44.98		3.46		1.01		6.89		2.35		4.82		10.18		47.03		36.84		37.72	
min			0.91		0.26		0.08		0.06		0.12		0.10		0.19		0.44		3.20		1.14		9.42	
SD			1.407		12.919		0.834		0.223		1.696		0.559		1.185		2.296		11.626		11.131		6.390	

obser-vation	geographical origin	year	Met	SD	Hyp	SD	Phe	SD	Cys	SD	Gln	SD	Orn	SD	Lys	SD	His	SD	Tyr	SD	Trp	SD	Σ (μg/kg)
			amino acid (%)																				
25	Amarante	2000	0.35	0.010	4.82	0.308	1.78	0.040	4.92	0.164	3.56	0.158	0.15	0.013	3.71	0.153	13.86	0.586	0.08	0.006	8.65	0.164	616.3
26		2001	0.08	0.008	11.58	0.607	0.61	0.007	1.98	0.085	2.04	0.042	0.17	0.002	0.86	0.025	1.82	0.001	0.18	0.001	0.35	0.004	793.6
27		2002	0.34	0.008	3.93	0.070	0.18	0.008	13.75	0.406	3.27	0.079	0.11	0.009	1.41	0.033	2.80	0.189	0.15	0.004	0.26	0.004	755.9
28	Baião	2000	0.02	0.001	9.51	0.205	0.75	0.014	1.90	0.025	1.49	0.034	0.33	0.005	2.72	0.054	2.99	0.054	0.31	0.005	0.23	0.005	1819.9
29		2001	0.48	0.014	5.60	0.098	0.24	0.013	8.07	0.301	1.73	0.071	0.11	0.003	1.39	0.047	1.63	0.022	0.19	0.008	0.17	0.007	461.4
30		2002	0.15	0.012	2.40	0.123	0.29	0.011	6.30	0.179	2.54	0.055	0.02	0.001	0.56	0.018	4.67	0.030	0.02	0.001	0.18	0.005	1861.8
31	Bragança	2000	0.13	0.001	13.36	0.872	0.40	0.026	2.71	0.119	0.80	0.021	0.07	0.001	1.78	0.063	3.63	0.074	0.17	0.010	0.92	0.056	1529.9
32		2001	0.18	0.008	4.54	0.466	0.06	0.001	1.04	0.045	0.52	0.037	0.40	0.014	1.14	0.041	7.66	0.356	0.20	0.005	0.06	0.004	454.1
33		2002	1.28	0.016	5.06	0.036	0.19	0.011	6.00	0.002	3.36	0.177	0.04	0.001	0.37	0.004	1.32	0.095	0.16	0.012	0.84	0.024	1213.2
34	Caminha	2001	0.14	0.006	13.14	0.324	0.16	0.004	1.45	0.015	0.97	0.031	0.01	0.001	0.95	0.040	0.89	0.038	0.01	0.001	0.35	0.011	1289.6
35	Covilhã	2000	0.36	0.016	10.97	0.524	0.11	0.007	4.84	0.359	0.21	0.005	0.09	0.005	0.83	0.063	0.38	0.005	0.04	0.001	0.07	0.002	700.1
36		2001	0.25	0.014	24.81	0.782	0.18	0.006	1.90	0.038	1.13	0.032	0.10	0.005	0.60	0.025	2.34	0.113	0.11	0.005	0.32	0.016	2334.6
37		2002	0.37	0.013	2.26	0.029	0.12	0.005	0.45	0.010	0.66	0.014	0.11	0.005	0.83	0.009	27.71	0.750	0.08	0.002	0.94	0.019	1816.8
38	Custóias	2001	0.16	0.004	14.11	0.703	0.25	0.006	1.27	0.036	2.03	0.080	0.24	0.005	0.56	0.019	1.68	0.005	0.28	0.012	0.18	0.002	1753.6
39		2002	0.16	0.016	7.06	0.237	0.13	0.006	0.33	0.004	0.82	0.041	0.12	0.005	1.95	0.038	19.25	0.409	0.08	0.003	0.54	0.011	1150.1
40	Pinhel	2000	0.03	0.004	3.78	0.073	0.48	0.019	1.92	0.084	0.20	0.013	0.23	0.004	0.95	0.032	3.95	0.104	0.05	0.001	0.42	0.004	874.1
41		2001	0.03	0.002	1.11	0.049	0.02	0.001	1.62	0.058	0.20	0.002	0.26	0.007	0.70	0.040	5.85	0.141	0.18	0.017	0.45	0.043	696.4
42		2002	0.61	0.001	2.70	0.098	0.14	0.006	0.33	0.001	0.42	0.002	0.07	0.001	1.10	0.004	22.55	0.531	0.05	0.001	0.96	0.021	930.4
43	Vila Real	2000	0.77	0.049	2.92	0.015	0.57	0.019	10.00	0.242	0.54	0.001	0.18	0.006	2.10	0.098	0.93	0.047	0.09	0.009	0.10	0.004	511.7
44		2001	0.02	0.002	8.97	0.511	0.36	0.004	1.19	0.028	1.72	0.032	0.09	0.004	1.23	0.024	5.81	0.204	0.05	0.003	0.47	0.011	903.5
45		2002	0.10	0.004	8.02	0.208	0.46	0.012	0.72	0.029	0.11	0.009	0.07	0.003	0.64	0.022	15.03	0.423	0.07	0.002	0.71	0.013	1484.3
46	Viseu	2000	0.14	0.012	7.77	0.224	0.11	0.004	1.21	0.016	1.91	0.055	0.75	0.015	1.01	0.014	5.01	0.177	0.10	0.004	0.64	0.035	1017.3
47		2001	0.03	0.002	14.14	1.084	0.15	0.001	0.80	0.030	1.29	0.009	0.02	0.002	0.65	0.007	6.35	0.117	0.06	0.001	1.17	0.022	1368.8
48		2002	0.34	0.015	5.08	0.027	0.46	0.008	9.02	0.362	3.03	0.114	0.10	0.001	1.41	0.054	3.26	0.091	0.18	0.008	0.45	0.015	771.2
mean			0.27		7.82		0.34		3.49		1.44		0.16		1.23		6.72		0.12		0.81		1129.5
max			1.28		24.81		1.78		13.75		3.56		0.75		3.71		27.71		0.31		8.65		2334.6
min			0.02		1.11		0.02		0.33		0.11		0.01		0.37		0.38		0.01		0.06		454.1
SD			0.289		5.449		0.361		3.617		1.087		0.159		0.772		7.424		0.079		1.699		518.05

[a] SD, standard deviation of three determinations; Σ, sum of the determined free amino acids; Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Ile, isoleucine; Pro, proline; Thr, threonine; Ser, serine; Glu, glutamic acid; Asn, asparagine; Asp, aspartic acid; Σ, sum of the determined free amino acids; Met, methionine; Hyp, hydroxyproline; Phe, phenylalanine; Cys, cysteine; Gln, glutamine; Orn, ornithine; Lys, lysine; His, histidine; Tyr, tyrosine; Trp, tryptophan.

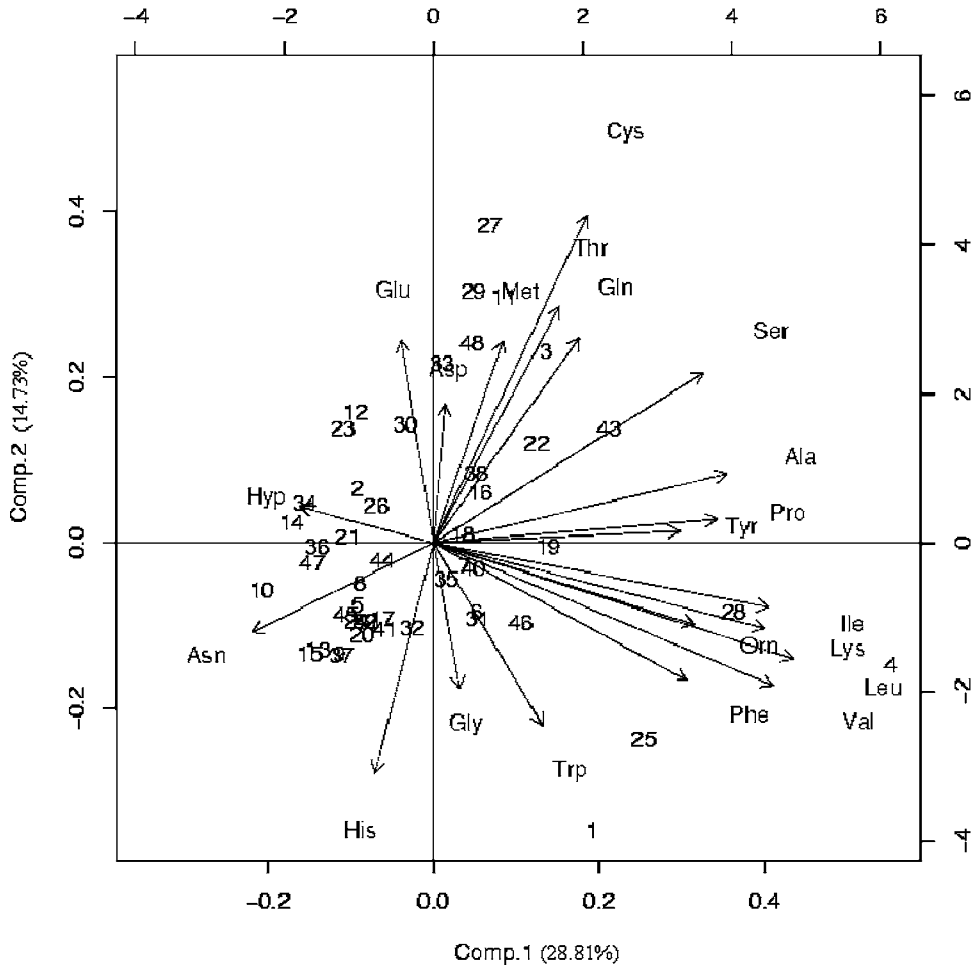

Figure 3. PCA of free amino acids in quince fruit, from 48 independent observations. Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Ile, isoleucine; Pro, proline; Thr, threonine; Ser, serine; Glu, glutamic acid; Asn, asparagine; Asp, aspartic acid; Met, methionine; Hyp, hydroxyproline; Phe, phenylalanine; Cys, cysteine; Gln, glutamine; Orn, ornithine; Lys, lysine; His, histidine; Tyr, tyrosine; Trp, tryptophan.

acid, and 2 kaempferol glycosides acylated with *p*-coumaric acid) (**Table 2**), as previously observed (*10*, *19*, *20*).

Generally, in quince pulp, the most abundant phenolic was 5-*O*-caffeoylquinic acid, whereas the major phenolic compound in quince peel was rutin. According to Silva et al. (*10*), in all studied cases, quince peel had a higher amount of phenolics than quince pulp. Absorption of UV light is a general feature of phenolic compounds (*26*). Some of them can be considered as filters that protect certain fragile cell structures (e.g., chloroplasts) from UV radiation. These filters consist mainly of flavonols and are located in the skins of fruits (*26*). In addition, because of their antioxidant properties, polyphenols can serve as protection against photooxidation caused by UV light (*26*). The antioxidant potential of quince pulp and peel methanolic extracts has already been reported (*20*). Peel methanolic extract exhibited greater antioxidant activity than the corresponding pulp extract, mainly due to the different qualitative and quantitative phenolic profile of these two parts of quince fruit.

The linear regression analysis (ANOVA full model) showed significant differences between the phenolic profiles of quince pulp and peel ($p < 0.001$). Significant differences were also found among the samples harvested in the three years, in terms of 3-*O*-caffeoylquinic acid ($p < 0.001$), 5-*O*-caffeoylquinic acid ($p < 0.05$) (only in pulps), and rutin ($p < 0.001$). Geographical origin did not influence significantly the phenolic composition of this fruit.

The differences between pulp and peel phenolic profiles were emphasized during PCA. Two main PCs accounted 81.29% of the total variability, PC1 (74.14%) and PC2 (7.15%) (**Figure 1**). PC1 is primarily responsible for the difference between the contents of caffeoylquinic acids (3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids and 3,5-*O*-dicaffeoylquinic acid) and flavonoids (quercetin 3-galactoside, rutin, kaempferol glycoside, kaempferol 3-glucoside, kaempferol 3-rutinoside, quercetin glycosides acylated with *p*-coumaric acid, and kaempferol glycosides acylated with *p*-coumaric acid). This characterizes the difference in the phenolic composition of pulp and peel. For example, quince pulp had an average content of 3-*O*-caffeoylquinic acid of 30.51 ± 10.058%, whereas peel had an average value of 11.77 ± 7.426%; peel had an average content of kaempferol-3-rutinoside of 3.95 ± 1.890%, whereas in pulp this flavonoid was absent (**Tables 1** and **2**). PC2 relates the content of 4-*O*-caffeoylquinic acid against the contents of 5-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids.

Generally, peel had a lower dispersion in terms of caffeoylquinic acids and flavonoids composition, making it possible to pool the data. However, the pulps had significant differences in the caffeoylquinic acids composition. Here, it is possible to observe three main groups: one rich in 4-*O*-caffeoylquinic acid and poor in 5-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids (observations 2, 10, and 24); another rich in 5-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids and poor in 4-*O*-caffeoylquinic acid (observations 1, 4, and 6); and another with average

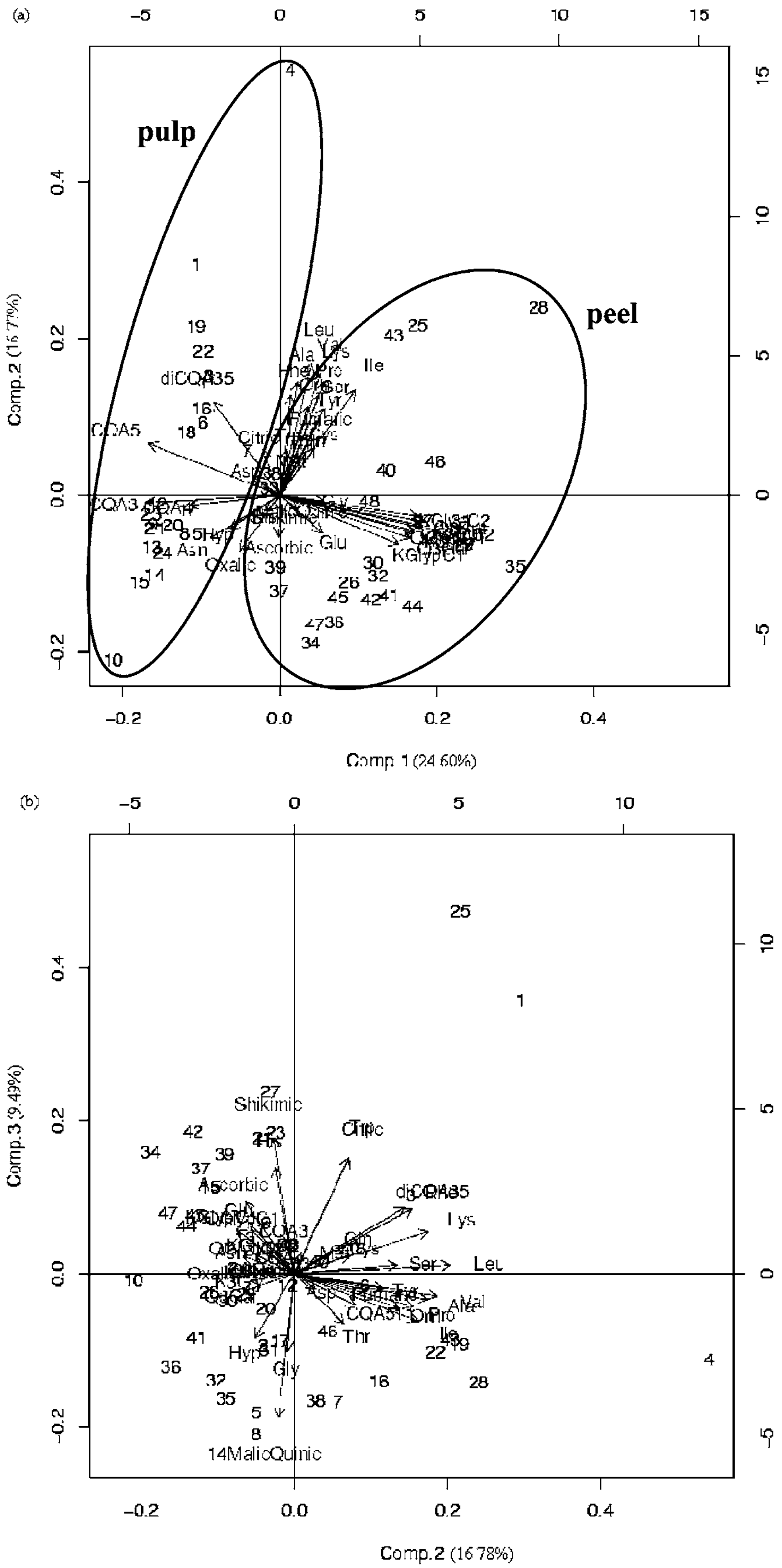

Figure 4. PCA of phenolics, organic acids, and free amino acids in quince fruit, from 48 independent observations: (**a**) PC1 versus PC2; (**b**) PC2 versus PC3. CQA3, 3-*O*-caffeoylquinic acid; CQA4, 4-*O*-caffeoylquinic acid; CQA5, 5-*O*-caffeoylquinic acid; diCQA35, 3,5-*O*-dicaffeoylquinic acid; Q3Gal, quercetin 3-galactoside; Q3Rut, rutin; K3Gly, kaempferol 3-glycoside; K3Glu, kaempferol 3-glucoside; K3Rut, kaempferol 3-rutinoside; QGly-pC1 and QGly-pC2, quercetin glycosides acylated with *p*-coumaric acid; KGly-pC1 and KGly-pC2, kaempferol glycosides acylated with *p*-coumaric acid; Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Ile, isoleucine; Pro, proline; Thr, threonine; Ser, serine; Glu, glutamic acid; Asn, asparagine; Asp, aspartic acid; Met, methionine; Hyp, hydroxyproline; Phe, phenylalanine; Cys, cysteine; Gln, glutamine; Orn, ornithine; Lys, lysine; His, histidine; Tyr, tyrosine; Trp, tryptophan.

composition (the rest of the observations), which may indicate the occurrence of caffeoylquinic acids isomerization in pulp matrix, once, according to Macheix et al. (*26*), transesterification of caffeoylquinic acids appears to be possible in fruit matrices.

From the food quality control point of view, it is very important to distinguish between quince pulp and peel, because Portuguese legislation (*1*) forbids the use of peel in the manufacture of quince jam.

Organic Acids. As previously reported (*11*), generally, the pulp and peel had similar profiles composed of seven identified organic acids: oxalic, citric, ascorbic, malic, quinic, shikimic, and fumaric acids (**Tables 3** and **4**). Quince fruit is characterized by large amounts of malic plus quinic acids, both in pulp and in peel, containing an average value of 96.45%, with maximum and minimum values of 99.46 and 90.46%, respectively. The ANOVA detected significant differences in the composition of quince fruits collected in the years 2000, 2001, and 2002, in terms of ascorbic acid ($p < 0.05$), shikimic acid ($p < 0.05$), fumaric acid ($p < 0.01$), and total organic acid content ($p < 0.001$), leading to the occurrence of a small decrease of organic acid total content for years 2000−2002. The part of the fruit and the geographical location did not influence significantly the organic acid composition of quince fruit.

Two PCs characterized the quince fruit organic acids composition (responsible for 61.61% of total variation). PC1 describes the domain of malic plus quinic acids on the quince fruit organic acid composition (37.54% of all variation). PC2 describes the orthogonality between oxalic plus ascorbic acids and citric acid in some quince fruits (24.07% of total variation). It is possible to observe that most samples presented large proportions of malic plus quinic acids, lowering the content of the other acids. **Figure 2** shows the high orthogonality between the oxalic plus ascorbic acids and citric acid. Some samples were very rich in terms of citric acid, with very low ascorbic and oxalic acids contents (observations 1 and 23), and others were rich in oxalic plus ascorbic acids but poor in citric acid (samples 15 and 44). It is also possible to observe some samples (3, 21, 27, 34, 39, and 42) balanced in terms of oxalic, ascorbic, and citric acids. In this case, in the PCA pulp and peel could not be distinguished.

Free Amino Acids. The quince fruit free amino acid profile was highly dispersed among the 21 constituents (**Tables 5** and **6** and **Figure 3**). Nevertheless, this fruit is richer in terms of asparagine (20.52%), aspartic acid (18.63%), glycine (12.32%), glutamic acid (11.66% for pulps and 18.01% for peels), hydroxyproline (11.64% for pulps and 7.82% for peels), and histidine (6.26%).

The ANOVA showed that some free amino acids contents vary significantly between harvesting year [Ala ($p < 0.05$), Val ($p < 0.001$), Leu ($p < 0.001$), Ile ($p < 0.001$), Pro ($p < 0.001$), Glu ($p < 0.001$), Phe ($p < 0.01$), Orn ($p < 0.05$), Lys ($p < 0.01$), and His ($p < 0.001$)] and geographical origin [Leu ($p < 0.05$), Lys ($p < 0.01$), and Tyr ($p < 0.05$)]. Generally, the free amino acids profiles are similar in pulp and peel. Nevertheless, the hydroxyproline content is significantly higher in pulp, whereas the glutamic acid content is significantly lower in this part of the fruit ($p < 0.05$).

The large dispersion in free amino acids composition led to a large number of PCs with significant variation ($n = 6$, >5% of the total variation). The first two PCs account for 43.54% of the total variability (28.81 and 14.73%, respectively) (**Figure 3**). PC1 represents the ratio of alanine, valine, leucine, isoleucine, proline, threonine, serine, phenylalanine, cysteine, glutamine, ornithine, lysine, and tyrosine against asparagine and hydroxyproline contents. PC2 describes the ratio of threonine, serine, glutamic acid, aspartic acid, methionine, cysteine, and glutamine against glycine, valine, leucine, asparagine, phenylalanine, ornithine, lysine, histidine, and tryptophan contents. **Figure 3** shows that all observations lie around the PC1 and PC2 center. The large variability of the free amino acids profile allows observations such as 4, 25, and 27, where the amounts of isoleucine, lysine, leucine, and valine (sample 4), phenylalanine and tryptophan (sample 25), and cysteine, threonine, methionine, and glutamic acid (sample 27) are unbalanced against the rest of the observations. In this case, in the PCA pulp and peel could not be distinguished.

Global Analysis. Figure 4 presents the PCs of quince fruit composition (phenolics, organic acids, and free amino acids). Correlation analysis shows that there was no direct correlation among phenolics, organic acids, and free amino acids, so they are considered as independent observations. Three PCs explain 50.86% of the variability of all data: PC1 (24.60%), PC2 (16.78%), and PC3 (9.49%). PC1 emphasizes the differences in terms of phenolic compounds between pulp and peel. PC2 presents the differences between caffeoylquinic acids and flavonoids composition of pulp and peel, as well as the small differences in organic acids and free amino acids. PC3 describes the variation in terms of organic acids and the orthogonality existent between 3,5-*O*-dicaffeoylquinic acid and 4-*O*-caffeoylquinic acid.

Conclusions. After the analysis of several samples of quince pulp and peel of quince fruits from nine geographical locations in Portugal, harvested in three consecutive years (2000−2002), it can be concluded that phenolics determination is the most interesting with regard to the discrimination of these two parts of the fruit. The content of organic acids is very characteristic of quince fruit (both pulp and peel), being dominated by malic and quinic acids, the sum of which represents always >90% of the organic acids total content. Among the chemical parameters analyzed, the free amino acids profile is the most variable. Nevertheless, quince pulp is characterized by higher hydroxyproline and lower glutamic acid contents than peel. These data may be useful for the elaboration of a database for the detection of adulteration in quince products.

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