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ORIGINAL PAPER

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Influence of jam processing upon the contents of phenolics, organic acids and free amino acids in quince fruit (*Cydonia oblonga* Miller)

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Abstract Phenolic compounds, organic acids and free amino acids of quince were evaluated, before and after jam processing, to test the effect of thermal processing in these compounds. In addition, the composition of jams prepared with peeled and unpeeled quinces was compared. Phenolics, organic acids and free amino acids were analysed by HPLC/DAD, HPLC/UV and GC/FID, respectively.

Keywords *Cydonia oblonga* Miller · Quince fruit · Jam · Phenolic compounds · Organic acids · Free amino acids

Introduction

In recent years it has become evident that significant health risks and benefits are associated with dietary food choice [1]. Nutritional studies recommend the regular consumption of fruits and vegetables, which constitute an essential part of the Mediterranean diet, to favour a healthy quality of life [2]. Among fruits, quince is an important source of health-promoting constituents, such as phenolics, organic acids and amino acids. Although fresh quince fruit is not edible when raw, because of its hardness, bitterness and astringency, it is very appreciated in Portugal for its jam. According to the Portuguese legislation [3], quince jam is the food product of the homogeneous and consistent mixture, obtained exclusively by boiling quince mesocarp with sugars.

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Some chemical studies have been developed on quince fruit and its derivatives. The usefulness of phenolic compounds in the determination of genuineness of quince puree [4], jam [5, 6]and jelly [7] has been reported. Glucosides of procyanidin polymers have been previously identified in this fruit [8, 9]. Recently, it has become possible to discriminate quince pulp and peel by the analysis of its phenolic compounds [10]. In 2002, an HPLC/UV method was developed for the determination of organic acids in quince fruit and jam [11]. More recently, a GC/FID method was developed for the determination of free amino acids in the same matrices [12].

The main purpose of this study was to investigate the influence of jam processing in quince composition, in terms of phenolic compounds, organic acids and free amino acids. As a sequence of previous studies on the distinction between quince pulp and peel [10, 11], we tested the possibility of detecting peel in quince jams by using the referred compounds. With this aim, two quince jams were also prepared and analysed, one of them from peeled quinces and another from unpeeled fruit.

Materials and methods

Samples

Healthy quince fruit were collected in Amarante (northern Portugal). Some fruit were separated into pulp and peel and each part of the fruit was cut into thin slices and freeze-dried. Lyophilisation was carried out using a Labconco 4.5 apparatus (Kansas City, MO). Other fruit were used to prepare quince jams.

A quince jam (jam A) was prepared in the laboratory by boiling fresh quince pulp with sugar (in the proportion of 50:50), for approximately 90 min. Another quince jam (jam B) was similarly prepared, but using unpeeled quinces.

Standards

The standards were from Sigma (St. Louis, MO, USA) and from Extrasynthése (Genay, France). Methanol, formic and hydrochloric

acids were obtained from Merck (Darmstadt, Germany) and sulphuric acid 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, USA).

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 (Mid Glamorgan, UK). The benzenesulfonic SCX Spe-ed SPE cartridges (200 mg; 3 mL) were obtained from Applied Separations (Allentown, USA).

Extraction of phenolic compounds

The extraction of phenolics was achieved as previously reported [6, 10] and included a C18 NEC SPE cleaning step.

Extraction of organic acids

The sample preparation was simple, involving only extraction with methanol (40 °C) and filtration through a C18 NEC SPE cartridge, as reported by Silva et al. [11].

Extraction of free amino acids

According to Silva et al. [12], the extraction of L-amino acids was simple, including a SCX SPE purification step.

Derivatisation procedure

The derivatisation of L-amino acids was carried out as reported previously [12].

HPLC analysis of phenolics

The extracts were analysed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0×0.46 cm; 5 μm, particle size) column [4, 5, 7, 10]. Detection was achieved with a Gilson DAD.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. 3- and 4-*O*-caffeoylquinic, and 3,5-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.

HPLC analysis of organic acids

The separation was carried out as previously reported [11] with an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel Ion 300 OA (300×7.7 mm) column. 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. The other acids were quantified as themselves.

GC analysis of free amino acids

The extracts were analysed on a Chrompack CP 9001 instrument (Chrompack, Middelburg, The Netherlands) equipped with a flame ionisation detector (FID), and an automatic liquid sampler (CP-9050, Chrompack) [12]. 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.

Results and discussion

Phenolic compounds

Quince pulp presented a chemical profile composed by six identified phenolic compounds: 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic and 3,5-dicaffeoylquinic acids, quercetin 3-galactoside and rutin. Quince peel contained thirteen phenolics: the six compounds presented in pulps, plus kaempferol 3-glucoside, kaempferol 3-rutinoside, and five not totally identified compounds (one kaempferol glycoside, two quercetin glycosides acylated with *p*-coumaric acid and two kaempferol glycosides acylated with *p*-coumaric acid). The samples now under study revealed the same phenolic composition as those collected in 2000 [10].

In pulp, caffeoylquinic acids represented 97% of the determined phenolics, with 3-*O*-caffeoylquinic acid being the most abundant (45%). Peel contained 66% of flavonol derivatives, with rutin being the major one (47%). Peel had a higher amount of phenolics than pulp (about 8 times) (Table 1).

In order to test if it is possible to detect quince peel in jams, two quince jams were prepared, one of them with peeled fruit (jam A) and the other one with unpeeled fruit (jam B). The total flavonoid content of jam A was 3%, while that of jam B was 19%. The amount of the total identified phenolics was duplicated in the jam that was prepared with unpeeled quinces (jam B) (Table 1). As expected, qualitatively, these two jams had different phenolic profiles: jam A, as quince pulp, presented a profile composed by 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic and 3,5-dicaffeoylquinic acids, quercetin 3-galactoside and rutin, while jam B contained these six compounds, plus kaempferol 3-glucoside, kaempferol 3-rutinoside, and the five not totally identified compounds found in quince peels. So, as previously reported [10], it seems that the phenolic profile determination allows the detection of adulterations in quince jams by addition of quince peel.

Although quince jam A had been prepared with 50% of pulp, its total phenolic content corresponded to about 57% of that of the used pulp. This could be due to evaporation during thermal processing. As in quince pulp, caffeoylquinic acids represented 97% of the determined phenolics in jam A. Nevertheless, the major compound (5-*O*-caffeoylquinic acid) was not the same, which may indicate the occurrence of isomerisation of caffeoylquinic acids .

Table 1 Phenolic composition of quince pulp, peel and jams (mg/kg). Quantification by external standard technique

Phenolic compounds	Samples							
	Pulp		Peel		Jam A		Jam B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3-CQA	94.6	0.74	209.9	4.61	43.1	0.34	87.3	5.97
4-CQA	8.5	0.01	19.2	0.62	8.7	0.28	17.0	0.60
5-CQA	90.0	1.08	279.8	8.61	58.4	0.46	84.6	3.53
3,5-diCQA	9.3	0.07	24.3	2.05	4.4	0.08	4.7	0.02
Q-3-gal	2.5	0.02	60.7	5.31	1.8	0.08	5.2	0.13
Q-3-rut	3.3	0.04	740.8	28.14	1.9	0.06	31.1	0.73
K-gly	nd	–	32.2	1.57	nd	–	0.9	0.15
K-3-glu	nd	–	25.8	0.40	nd	–	0.7	0.04
K-3-rut	nd	–	51.0	1.13	nd	–	3.0	0.09
Q-gly-*p*-CouA1	nd	–	52.0	0.92	nd	–	1.3	0.05
Q-gly-*p*-CouA2	nd	–	17.7	0.05	nd	–	0.6	0.02
K-gly-*p*-CouA1	nd	–	18.4	0.10	nd	–	0.9	0.03
K-gly-*p*-CouA2	nd	–	34.5	0.19	nd	–	1.0	0.15
Σ	208.1		1566.4		118.3		238.3	
HMF	nd	–	nd	–	503.9	5.52	203.0	1.64

Values are expressed as mean of three determinations
Jam A is quince jam prepared with peeled fruit, Jam B is quince jam prepared with unpeeled fruit
SD standard deviation, Σ sum of the determined phenolic compound, *nd* not detected
3-CQA 3-*O*-caffeoylquinic acid, *4-CQA* 4-*O*-caffeoylquinic acid, *5-CQA* 5-*O*-caffeoylquinic acid, *3,5-diCQA* 3,5-dicaffeoylquinic acid, *Q-3-Gal* quercetin 3-galactoside, *Q-3-Rut* rutin, *K-Gly* kaempferol glycoside, *K-3-Glu* kaempferol 3-glucoside, *K-3-Rut* kaempferol 3-rutinoside, *Q-gly-p-CouA1* and *Q-gly-p-CouA2* quercetin glycosides acylated with *p*-coumaric acid, *K-gly-p-CouA1* and *K-gly-p-CouA2* kaempferol glycosides acylated with *p*-coumaric acid, *HMF* hydroxymethylfurfural

Table 2 Organic acid composition of quince pulp, peel and jams (mg/kg). Quantification by external standard technique

Organic acids	Samples							
	Pulp		Peel		Jam A		Jam B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oxalic acid	9.1	0.01	11.5	0.20	6.1	0.04	2.6	0.05
Citric acid	287.5	2.81	294.3	13.88	54.0	2.02	42.1	3.56
Ascorbic acid	159.0	6.63	189.4	9.77	34.4	0.80	49.1	1.69
Malic+quinic acids	6936.2	30.12	6706.9	381.08	4457.8	144.12	4321.3	69.38
Shikimic acid	24.3	0.08	26.5	0.07	4.2	0.12	8.0	0.21
Fumaric acid	tr	–	tr	–	tr	–	tr	–
Σ	7416.1		7228.6		4556.4		4423.0	

Values are expressed as mean of three determinations
Jam A is quince jam prepared with peeled fruit, Jam B is quince jam prepared with unpeeled fruit
SD standard deviation, Σ sum of the determined organic acids, *tr* traces

In chromatograms of quince jams (data not shown) it was possible to observe a peak corresponding to hydroxymethylfurfural (HMF). The presence of this compound is not strange as it results from sugar decomposition by heat during cooking.

Organic acids

All samples (pulp, peel and jams) presented a similar profile composed of seven identified organic acids: oxalic, citric, ascorbic, malic, quinic, shikimic and fumaric acids.

In quince pulp and peel, the sum of malic acid plus quinic acid represented 93% and all other acids were present in very small amounts, less than 0.5%, with the exceptions of citric and ascorbic acids (about 4 and 2%, respectively). The sum of all quantified acids was approximately 7 g/kg (Table 2), both in pulp and peel, which is in agreement with results previously reported [11].

Due to the similarity between the organic acid profile of pulp and peel, it was not possible, by using this chemical parameter, to differentiate these two parts of the fruit or the jams prepared with peeled or unpeeled fruit.

On comparing the total amount of organic acids in quince jam A with that of the used pulp, it can be seen that it corresponded to approximately 60%, despite being prepared with 50% of pulp. This fact can, probably, be explained by the occurrence of evaporation during jam processing.

In jams, the sum of malic acid plus quinic acid represented 98% and all other acids were present in very small amounts, less than 1.2%. It seems that the high temperatures used during processing caused some destruction of citric, ascorbic and shikimic acids.

Table 3 Free amino acids composition of quince pulp, peel and jams (μg/kg). Quantification by internal standard technique

Amino acids	Samples							
	Pulp		Peel		Jam A		Jam B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alanine	16.2	0.38	12.3	0.25	16.1	0.77	23.2	0.80
Glycine	1.4	0.05	3.5	0.15	9.4	0.23	9.1	0.20
Valine	4.1	0.16	1.7	0.27	5.1	0.37	4.7	0.23
Leucine	0.6	0.02	1.0	0.02	2.1	0.08	2.8	0.03
Isoleucine	11.8	0.31	6.9	0.37	2.9	0.09	5.0	0.11
Proline	2.0	0.09	4.7	0.13	3.6	0.10	4.6	0.03
Threonine	10.0	0.41	15.6	0.53	35.9	0.74	45.3	1.67
Serine	33.8	0.36	77.0	0.44	16.7	0.83	30.8	1.10
Glutamic acid	104.9	4.76	355.5	10.17	6.0	0.24	12.6	0.20
Asparagine	22.8	0.73	8.6	0.57	160.1	1.26	112.3	1.52
Aspartic acid	138.5	4.92	71.2	1.75	383.4	3.13	850.7	12.15
Methionine	1.2	0.04	2.5	0.06	1.1	0.02	0.2	0.01
Hydroxyproline	38.2	1.33	29.7	0.53	58.7	0.94	67.9	2.82
Phenylalanine	1.2	0.11	1.4	0.06	2.0	0.05	1.5	0.19
Cysteine	46.9	0.68	103.9	3.07	21.7	1.14	51.7	1.73
Glutamine	12.8	0.33	24.7	0.60	4.4	0.22	7.9	0.07
Ornithine	2.2	0.02	0.8	0.07	3.8	0.05	1.2	0.01
Lysine	8.1	0.18	10.7	0.25	5.4	0.13	8.6	0.20
Histidine	20.1	0.73	21.2	1.43	2.3	0.15	3.1	0.10
Tyrosine	1.3	0.04	1.1	0.03	2.1	0.04	3.4	0.06
Tryptophan	2.2	0.02	2.0	0.03	0.1	0.00	0.1	0.01
Σ	480.4		755.9		742.7		1246.5	

Values are expressed as mean of three determinations
Jam A is quince jam prepared with peeled fruit, Jam B is quince jam prepared with unpeeled fruit
SD standard deviation, Σ sum of the determined free amino acids

Free amino acids

As expected [12], all samples (quince fruits and jams) presented a similar qualitative profile composed of 21 identified free amino acids. Peel had a higher amount of total free amino acids than pulp (about 1.6 times) (Table 3). The five most abundant free amino acids were: aspartic and glutamic acids, cysteine, serine and hydroxyproline, and corresponded to about 75% and 85% of the totality of free amino acids of pulp and peel, respectively.

The five major free amino acids in jams were aspartic acid, asparagine, hydroxyproline, cysteine and threonine, that together accounted for about 90% of the totality of free amino acids (Table 3). Proline, hydroxyproline, phenylalanine, ornithine and tyrosine percentages seemed to be characteristic of pulp, since they stayed approximately constant in jam A.

Quince jams had higher total free amino acid content than pulp and peel (Table 3), which could be due to hydrolysis of proteins, peptides or other compounds with amino acids in their constitution, which can occur during thermal processing (in acid medium). Glutamic acid, histidine and tryptophan were present in much lower amounts in jams than in pulp or peel, which can be due to their thermolability. These amino acids can also take an active part in Maillard reactions and/or in browning processes after the enzymatic oxidation of polyphenols.

In conclusion, although jam processing leads to caffeoylquinic acid isomerisation and to the degradation of some organic acids (citric, ascorbic and shikimic acids), the total contents of the determined phenolics and organic acids in quince fruit and jam did not indicate appreciable changes. The free amino acid profile was changed by thermal processing, probably due to hydrolysis of amino acid-derived compounds.

The determination of the phenolic profile also allowed detection of quince peel in the jam prepared with unpeeled fruit, but organic acids and free amino acids profiles were not suitable for this purpose.

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