Quince (Cydonia oblonga Miller) Fruit (Pulp, Peel, and Seed) and Jam: Antioxidant Activity

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · AUGUST 2004

Impact Factor: 2.91 · DOI: 10.1021/jf040057v · Source: PubMed

6 AUTHORS, INCLUDING:

Branca Maria Silva
Universidade da Beira Interior
101 PUBLICATIONS **1,878** CITATIONS
SEE PROFILE

Federico Ferreres
Spanish National Research Council
297 PUBLICATIONS **8,819** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quince (*Cydonia oblonga* Miller) Fruit (Pulp, Peel, and Seed) and Jam: Antioxidant Activity

BRANCA M. SILVA,[†] PAULA B. ANDRADE,[†] PATRÍCIA VALENTÃO,[†] FEDERICO FERRERES,[‡] ROSA M. SEABRA,*[,†] AND MARGARIDA A. FERREIRA[§]

REQUIMTE, Serviço de Farmacognosia and Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 4050-047 Porto, Portugal, and Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Science and Technology, CEBAS-CSIC, P.O. Box 164, E-30100 Espinardo, Murcia, Spain

To study the antioxidant activity of quince fruit (pulp, peel, and seed) and jam, methanolic extracts were prepared. Each extract was fractionated into a phenolic fraction and an organic acid fraction and was analyzed by high-performance liquid chromatography (HPLC)/diode array detection and HPLC/UV, respectively. Antiradical activities of the extracts and fractions were evaluated by a microassay using 1,1′-diphenyl-2-picrylhydrazyl. The phenolic fraction always exhibited a stronger antioxidant activity than the whole methanolic extract. Organic acid extracts were always the weakest in terms of antiradical activity, which seems to indicate that the phenolic fraction gives a higher contribution for the antioxidant potential of quince fruit and jam. The evaluation of the antioxidant activity of methanolic extracts showed that peel extract was the one presenting the highest antioxidant capacity. The IC_{50} values of quince pulp, peel, and jam extracts were correlated with the caffeoylquinic acids total content. Among the phenolic fractions, the seed extract was the one that exhibited the strongest antioxidant activity. The IC_{50} values of quince pulp, peel, and jam phenolic extracts were strongly correlated with caffeoylquinic acids and phenolics total contents. For organic acid fractions, the peel extract was the one that had the strongest antiradical activity. The IC_{50} values of quince pulp, peel, and jam organic acid fractions were correlated with the ascorbic acid and citric acid contents.

KEYWORDS: ***Cydonia oblonga* Miller; phenolic compounds; organic acids; antioxidant activity; DPPH assay**

INTRODUCTION

In recent years, it has become evident that significant health risks and benefits are associated with dietary food choice (*1*). Fruits and vegetables are rich sources of vitamins, most notably vitamins A and C, and excellent sources of fiber, contain some calories, and are naturally low in fat (*2*). An increased consumption of fruits and vegetables has been associated with protection against various diseases, including cancers and cardio- and cerebrovascular diseases (*3*). This association is often attributed to the antioxidants present in the fruits and vegetables, such as vitamins C and E, carotenoids, phenolic acids, and flavonoids, which prevent free radical damage (*2*).

Quince fruit (*Cydonia oblonga* Miller, Rosaceae family) is a pome with numerous seeds. The fruits are big (10−12 cm in diameter), with variable dimensions and asymmetric shapes, and exhibit a characteristic fragrance. The peel is covered by an abundant hair, which disappears with fruit ripening. The white−yellow pulp, easily oxidized to air exposition, is firm, generally acidic, and astringent; so, it is not suitable for consumption when raw. The most important utilization of this fruit is in the production of jams and jellies, which are very appreciated in Portugal.

Several analytical methods were developed to determine phenolics, organic acids, and free amino acids in quince fruit and jams, and their composition, in terms of these compounds, was established (*4*−*11*). Among these parameters, the phenolic profile determination was revealed to be the most useful in the discrimination of the different parts of quince fruit (pulp, peel, and seed) (*7*, *9*) and in the evaluation of the genuineness of quince puree (*4*), jam (*5*, *6*), and jelly (*12*). Recently, the influence of jam processing upon the contents of phenolics, organic acids, and free amino acids in quince fruit was also evaluated (*13*).

The antioxidant activity of several fruits has been observed in different experimental models (*2*, *14*−*22*). García-Alonso et al. (*22*) analyzed 28 different fruits, including quince pulp, for antioxidant activity determination. Additionally, these authors tried to correlate the antioxidant activity and the flavanol content of these fruits. However, information concerning the antioxidant

* To whom correspondence should be addressed. Tel: 351 222078934. Fax: 351 222003977. E-mail: rseabra@ff.up.pt.
[†] Serviço de Farmacognosia, Universidade do Porto.
[‡] CEBAS-CSIC.
[§] Serviço de Bromatologia, Universidade do Porto.

10.1021/jf040057v CCC: $27.50
Published on Web 07/03/2004

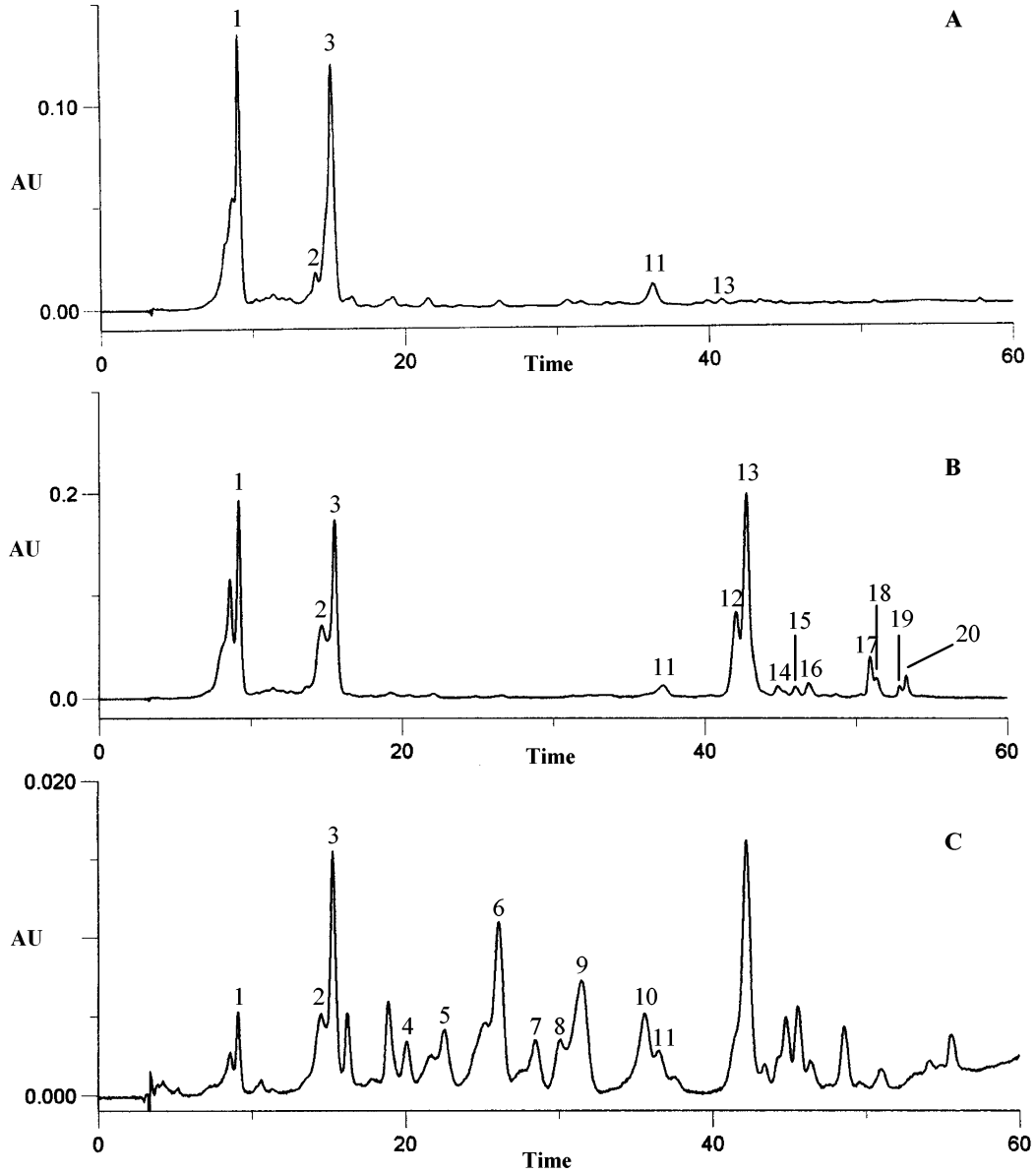


Figure 1. HPLC phenolic profile of the quince pulp (**A**), peel (**B**), and seed (**C**). Detection at 350 nm. Peaks: 1, 3-*O*-caffeoylquinic acid; 2, 4-*O*-caffeoylquinic acid; 3, 5-*O*-caffeoylquinic acid; 4, lucenin-2; 5, vicenin-2; 6, stellarin-2; 7, isoschaftoside; 8, schaftoside; 9, 6-C-pentosyl-8-C-glucoside of chrysoeriol; 10, 6-C-glucosyl-8-C-pentoside of chrysoeriol; 11, 3,5-dicaffeoylquinic acids; 12, quercetin 3-galactoside; 13, rutin; 14, kaempferol glycoside; 15, kaempferol 3-glucoside; 16, kaempferol 3-rutinoside; 17 and 18, quercetin glycosides acylated with *p*-coumaric acid; 19 and 20, kaempferol glycosides acylated with *p*-coumaric acid.

potential of quince peels, seeds, and jams is not available. So, in the sequence of previous works and regarding its chemical composition, the purpose of this study was to evaluate the antioxidant potential of quince fruits (pulp, peel, and seed) and jams. To accomplish this aim, the scavenging effect of quince fruit (pulp, peel, and seed) and jam methanolic extracts on 1,1′-diphenyl-2-picrylhydrazyl (DPPH) was studied. The antioxidant activity exhibited by the extracts will be the result of the action of different antioxidant compounds (even from distinct chemical classes) present, with synergies or antagonisms. Considering this, the methanolic extracts were fractionated into the phenolics fractions and the organic acids fractions, which were analyzed by high-performance liquid chromatography (HPLC)/diode array detection (DAD) and HPLC/UV, respectively, and their antioxidant activity was also evaluated. Correlations between the antiradical observed effect and the phenolics and organic acids content were made.

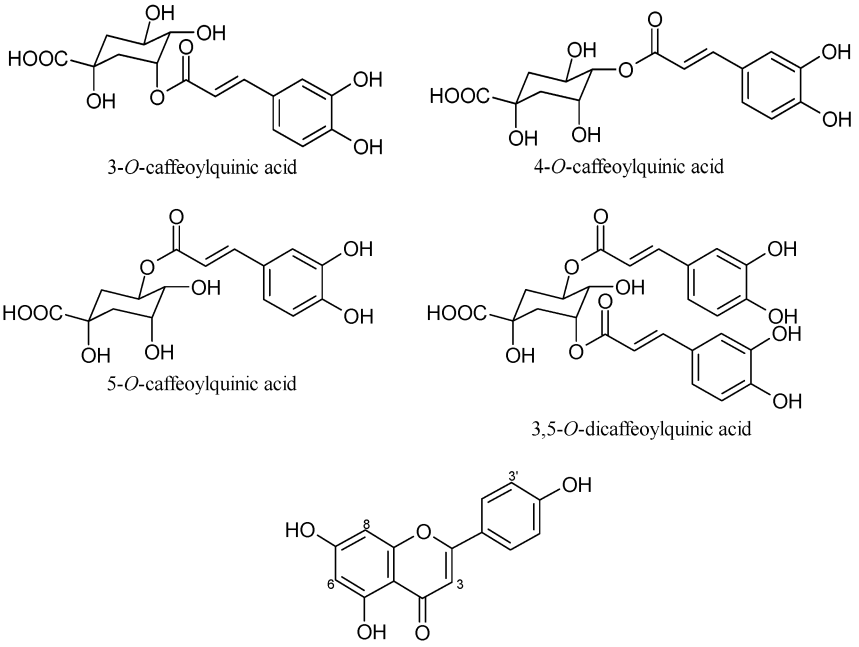
MATERIALS AND METHODS

Samples. Healthy quince fruits were collected in Amarante (Northern Portugal). Some fruits were separated into pulps, peels, and seeds, and each part was freeze-dried. Lyophilization was carried out using a Labconco 4.5 apparatus (Kansas City, MO). Other fruits were used to prepare quince jams.

One quince jam sample (jam A) was prepared in the laboratory by boiling fresh quince pulps 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) and from Extrasynthése (Genay, France). Methanol, formic, and hydrochloric acids were obtained from Merck (Darmstadt, Germany), and sulfuric acid was obtained from Pronalab (Lisboa, Portugal). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA). DPPH was from Sigma.

Solid Phase Extraction (SPE) Columns. The ISOLUTE C18 nonend-capped (NEC) SPE columns (50 μm particle size, 60 Å porosity;



Compound	3	6	8	3'
Quercetin 3-galactoside	O-Galactose	H	H	OH
Rutin	O-Rutinose	H	H	OH
Kaempferol 3-glucoside	O-Glucose	H	H	H
Kaempferol 3-rutinoside	O-Rutinose	H	H	H
Vicenin-2	H	Glucose	Glucose	H
Isoschaftoside	H	Arabinose	Glucose	H
Schaftoside	H	Glucose	Arabinose	H
Lucenin-2	H	Glucose	Glucose	OH
Stellarin-2	H	Glucose	Glucose	OCH_3
6-*C*-pentosyl-8-*C*-glucoside of chrysoeriol	H	Pentose	Glucose	OCH_3
6-*C*-glucosyl-8-*C*-pentoside of chrysoeriol	H	Glucose	Pentose	OCH_3

Figure 2. Phenolic compounds of quince fruit and jam.

10 g sorbent mass/70 mL reservoir volume) were purchased from International Sorbent Technology Ltd. (Mid Glamorgan, United Kingdom).

Methanolic Extracts. Each sample (ca. 1 g for lyophilized pulps, peels, and seeds and 5 g for jams) was thoroughly mixed with methanol (3 × 25 mL) (40 °C). The methanolic extract was filtered, concentrated to dryness under reduced pressure (40 °C) (the extraction efficiency in relation to fresh matter was variable as follows: 17, 14, 10, 89, and 73% for pulp, peel, seed, jam A, and jam B methanolic extracts), and redissolved in methanol (1 mL). These solutions were used for the DPPH assay.

Organic Acids Fractions. Each sample (ca. 1 g for lyophilized pulps, peels, and seeds and 5 g for jams) was thoroughly mixed with methanol (3 × 25 mL) (40 °C). The methanolic extract was filtered, concentrated to dryness under reduced pressure (40 °C), and redissolved in acidic water (pH 2.0 with HCl) (ca. 25 mL). The aqueous solutions obtained were passed through an ISOLUTE C18 (NEC) column, previously conditioned with 30 mL of methanol and 70 mL of acidic water (pH 2.0 with HCl). The aqueous extracts were evaporated to dryness under reduced pressure (40 °C) (ca. 30 min) and redissolved in acidic water (1 mL). These extracts were used for the organic acids analysis and DPPH assay.

Phenolic Compounds Fractions. After the elution of organic acids and other polar compounds with aqueous solvent, the retained phenolic fraction was eluted with methanol (ca. 50 mL). The extracts were concentrated to dryness under reduced pressure (40 °C) and redissolved in methanol (2 mL). These extracts were used for the phenolic compounds analysis and DPPH assay.

HPLC Analysis of Organic Acids. The separation was carried out as previously reported (*8*) with an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel Ion 300 OA (300 mm × 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.

HPLC Analysis of Phenolics. The extracts (20 μL) were analyzed as previously described (*4*−*7*, *12*, *13*), on an analytical HPLC unit (Gilson), using an Spherisorb ODS2 column (25.0 cm × 0.46 cm; 5 μm, particle size). 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.

DPPH Method. The antiradical activity of the extracts was determined spectrophotometrically in an ELX808 IU Ultra Microplate Reader (Bio-Tek Instruments, Inc.) by monitoring the disappearance of DPPH at 515 nm, according to a described procedure of Fukumoto and Mazza (*23*), although some modifications were made to the original DPPH method.

For each extract, a dilution series (five different concentrations) was prepared in a 96 well plate. The reaction mixtures in the sample wells consisted of 25 μL of extract and 200 μL of 150 mM DPPH (dissolved in methanol). The reaction was conducted at room temperature, until

Table 1. Phenolic Composition of Quince Pulp, Peel, Seed, and Jams Extracts (mg of Phenolic Compound kg^{-1} of Methanolic Extract Dry Matter)[a]

	samples									
	pulp		peel		seed		jam A		jam B	
phenolic compounds	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
3-CQA	684.6	18.86	1966.4	23.25	24.0	0.70	31.4	0.05	125.7	2.33
4-CQA	79.7	1.13	174.4	1.41	27.6	1.26	19.3	0.22	21.4	1.47
5-CQA	648.8	13.62	1829.4	31.00	54.4	1.56	72.7	0.47	128.1	0.71
lucenin-2	ND		ND		10.2	1.43	ND		ND	
vicenin-2	ND		ND		14.6	0.41	ND		ND	
stellarin-2	ND		ND		46.6	1.01	ND		ND	
isoschaftoside	ND		ND		17.1	0.04	ND		ND	
schaftoside	ND		ND		11.4	0.25	ND		ND	
6-C-pentosyl-8-C-glucoside of chrysoeriol	ND		ND		21.8	0.12	ND		ND	
6-C- glucosyl-8-C-pentoside of chrysoeriol	ND		ND		16.1	0.54	ND		ND	
3,5-diCQA	56.3	2.60	98.7	2.41	29.9	0.17	5.4	0.23	7.5	0.17
Q-3-gal	NQ		491.2	18.86	ND		NQ		8.7	0.43
Q-3-rut	33.0	2.37	1777.8	27.66	ND		5.3	0.33	48.8	0.32
K-gly	ND		112.2	0.72	ND		ND		3.4	0.27
K-3-glu	ND		88.8	2.49	ND		ND		2.2	0.21
K-3-rut	ND		152.2	1.51	ND		ND		3.1	0.12
Q-gly-*p*-CouA1	ND		166.9	1.29	ND		ND		3.0	0.06
Q-gly-*p*-CouA2	ND		65.7	0.26	ND		ND		1.1	0.03
K-gly-*p*-CouA1	ND		53.8	1.56	ND		ND		1.3	0.01
K-gly-*p*-CouA2	ND		109.5	4.77	ND		ND		2.8	0.21
Σ	1502.4		7087.1		273.6		134.1		357.1	
HMF	ND		ND		ND		836.8	9.16	632.0	7.25

[a] Values are expressed as means of three determinations; SD, standard deviation; Σ, sum of the determined phenolic compound; ND, not detected; NQ, not quantified; jam A, quince jam prepared with peeled fruits; jam B, quince jam prepared with unpeeled fruits; 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.

no variation of the absorbance was observed. Ascorbic acid was used as the reference compound. Four experiments were performed in triplicate.

The antiradical activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC_{50}). The IC_{50} value for each extract was determined graphically by plotting the percentage of DPPH scavenging as a function of extract concentration.

RESULTS AND DISCUSSION

Fruits and vegetables are one of the main sources of antioxidants in our diets (*2*, *14*−*22*). Our previous studies showed that quince fruit is a good source of phenolic acids, flavonoids, and organic acids (*4*−*9*, *12*, *13*), which are considered potent antioxidants (*2*). To test the antioxidant activities of quince fruits and jams, we prepared methanolic extracts of pulps, peels, seeds, and two jams, one of them prepared with peeled quinces (jam A) and another with unpeeled fruits (jam B).

Identification and Quantification of Phenolic Compounds by HPLC/DAD. Quince pulp and jam A extracts presented a chemical profile composed of six identified phenolic compounds: 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, and 3,5-dicaffeoylquinic acids; quercetin 3-galactoside; and rutin (**Figures 1A** and **2**), which is in accordance with previous studies (*7*, *13*). Quince peel and jam B extracts contained 13 phenolics: the six compounds presented in pulp and jam A extracts plus kaempferol 3-glucoside, kaempferol 3-rutinoside (**Figures 1B** and **2**), 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), as was already observed (*7*, *13*). Like previously described (*9*), the seed extract had a different composition, presenting the referred caffeoylquinic acids plus several flavone *C*-glycosides characteristic of this part of the fruit: lucenin-2, vicenin-2, stellarin-2, isoschaftoside, schaftoside, 6-C-pentosyl-8-C-glucoside of chrysoeriol, and 6-C-glucosyl-8-C-pentoside of chrysoeriol (**Figures 1C** and **2**). In the pulp extract, caffeoylquinic acids represented 98% of the determined phenolics, with 3-*O*-caffeoylquinic acid being the most abundant (46%), while peel extract contained 57% of flavonol derivatives, with rutin being the major one (25%). The peel extract had a higher amount of phenolics than that of the pulp (about five times) (**Table 1**). Caffeoylquinic acids represented 50% of the determined phenolics of seed extract, with 5-*O*-caffeoylquinic acid being the most abundant (20%). This extract contained 50% of flavone C-glycosides, and the major one was stellarin-2 (ca. 17%).

The total flavonoid content of jam A extract was 4%, while that of the jam B was 21% (**Table 1**), a fact that may be explained by the high flavonoidic content of the peel, which was not removed for the preparation of jam B. In quince jam extract chromatograms at 280 nm (data not shown), it was possible to observe a peak corresponding to hydroxymethylfurfural (HMF). The presence of this compound is not strange, once it results from sugar decomposition by heating and cooking duration.

Identification and Quantification of Organic Acids by HPLC/UV. As previously reported (*8*), pulp, peel, and jam extracts presented a similar profile composed of seven identified organic acids: oxalic, citric, ascorbic, malic, quinic, shikimic, and fumaric acids (**Figures 3** and **4**). Oxalic acid was the only compound that was not detected in seed extract.

In pulp, peel, and jam extracts, the sum of malic acid plus quinic acid always represented at least 95% of the organic acid content and all other acids were present in very small amounts (**Table 2**). The seed extract was very distinct from the others, in which the sum of malic acid plus quinic acid represented only 33% of the total content (**Table 2**). Citric and ascorbic

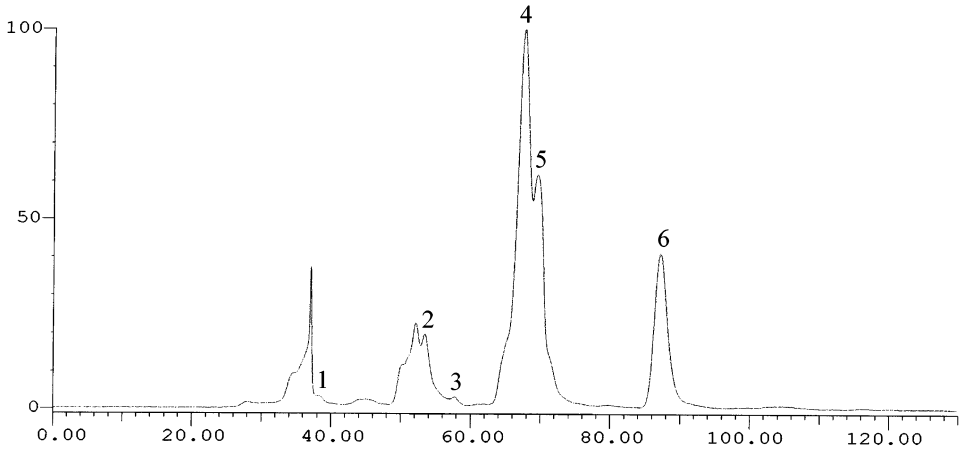


Figure 3. HPLC organic acid profile of the quince peel. Detection at 214 nm. Peaks: 1, oxalic acid; 2, citric acid; 3, ascorbic acid; 4, malic acid; 5, quinic acid; 6, shikimic acid.

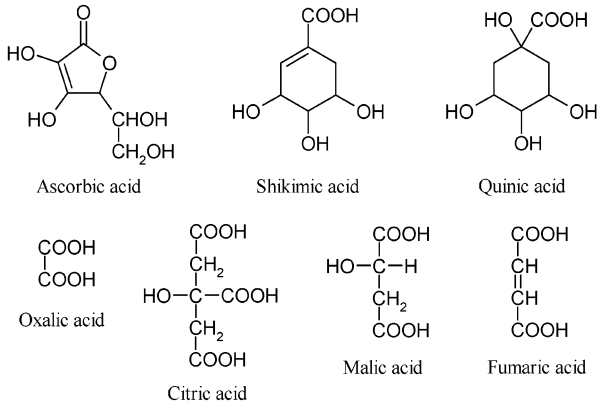


Figure 4. Organic acids of quince fruit and jam.

acids were also present in great percentages (36 and 31%, respectively). The organic acid total content of seed extract was the lowest.

Antioxidant Activity Determination by DPPH Assay. Once methanolic and acid water extracts (pH 2.0 with HCl) were used, it was necessary to determine the IC_{50} of ascorbic acid solutions (1 mg mL^{-1}), dissolved in methanol and acidic water (pH 2.0 with HCl). The ascorbic acid solution IC_{50} value, 5.6 μg mL^{-1}, was not affected by the solvent.

In what concerns the antioxidant activities of methanolic extracts, the peel extract was the one that showed the strongest antioxidant activity (IC_{50} of 0.6 mg mL^{-1}), followed by pulp and seed extracts, with very similar activities (IC_{50} of 1.7 and 2.0 mg mL^{-1}, respectively) (**Table 3** and **Figure 5A**). Jam A and B extracts also had similar antiradical activities (IC_{50} of 8.9 and 8.4 mg mL^{-1}, respectively) (**Table 3** and **Figure 5B**). The results obtained seem to indicate that the IC_{50} of quince pulp, peel, and jam methanolic extracts is correlated with the caffeoylquinic acids total content (exponential decay; $r = 0.99350$; $p < 0.05$). The seed extract exhibited a different behavior, probably because of its different composition, in terms of phenolics (presence of flavone C-glycosides and absence of flavonols) and in terms of organic acids (different individual organic acids percentages and lower organic acid total content).

Among the phenolic extracts, the seed extract was the one that showed the strongest antioxidant activity (IC_{50} of 0.1 mg mL^{-1}), followed by the peel extract with an IC_{50} of 0.4 mg mL^{-1} and the pulp extract with an IC_{50} of 1.0 mg mL^{-1} (**Table 3** and **Figure 6A**). Jam A and B extracts had similar antiradical activities (IC_{50} of 7.0 and 6.0 mg mL^{-1}, respectively) (**Figure 6B**). The IC_{50} values of quince pulp, peel, and jam phenolic extracts were strongly correlated with the caffeoylquinic acids

Table 3. IC_{50} Values (mg mL^{-1}), Phenolics, and Organic Acids Total Contents (mg kg^{-1}) of Quince Pulp, Peel, Seed, and Jams Extracts[a]

	methanolic extract	phenolics fraction		organic acids fraction	
samples	IC_{50}	total content	IC_{50}	total content	IC_{50}
pulp	1.7	1502.4	1.0	16623.7	11.6
peel	0.6	7087.1	0.4	14440.5	6.9
seed	2.0	273.6	0.1	1858.0	12.9
jam A	8.9	134.1	7.0	4014.0	22.6
jam B	8.4	357.1	6.0	4234.9	16.3

[a] Jam A, quince jam prepared with peeled fruits; jam B, quince jam prepared with unpeeled fruits.

Table 2. Organic Acids Composition of Quince Pulp, Peel, Seed, and Jams Extracts (mg of Organic Acid kg^{-1} of Methanolic Extract Dry Matter)[a]

	samples									
	pulp		peel		seed		jam A		jam B	
organic acids	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
oxalic acid	NQ		5.2	0.01	ND		7.2	0.01	NQ	
citric acid	159.0	1.11	378.2	15.17	670.2	18.76	53.8	0.24	78.3	5.97
ascorbic acid	109.1	0.06	187.4	7.47	567.9	16.03	27.5	0.01	52.5	0.01
malic + quinic acids	16310.3	176.94	13818.1	66.97	611.8	7.98	3921.3	272.76	4094.1	214.54
shikimic acid	45.3	0.24	51.6	0.04	4.2	0.16	4.2	0.86	10.0	0.24
fumaric acid	NQ		NQ		3.9	0.06	NQ		NQ	
Σ	16623.7		14440.5		1858.0		4014.0		4234.9	

[a] Values are expressed as means of three determinations. SD, standard deviation; Σ, sum of the determined organic acids; NQ, not quantified; ND, not detected; jam A, quince jam prepared with peeled fruits; jam B, quince jam prepared with unpeeled fruits.

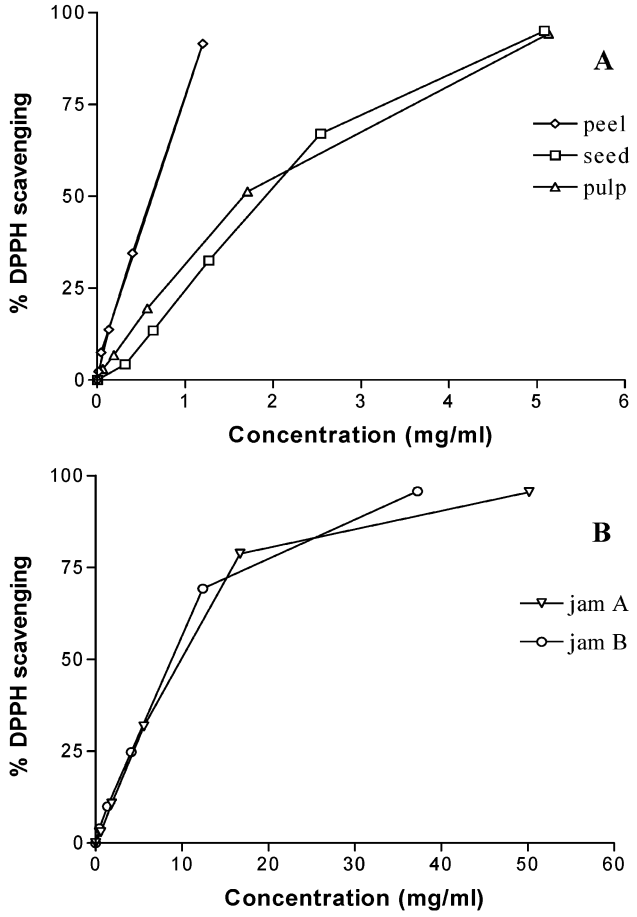


Figure 5. Antiradical activity of quince pulp, peel, and seed (**A**) and jams A and B (**B**) methanolic extracts.

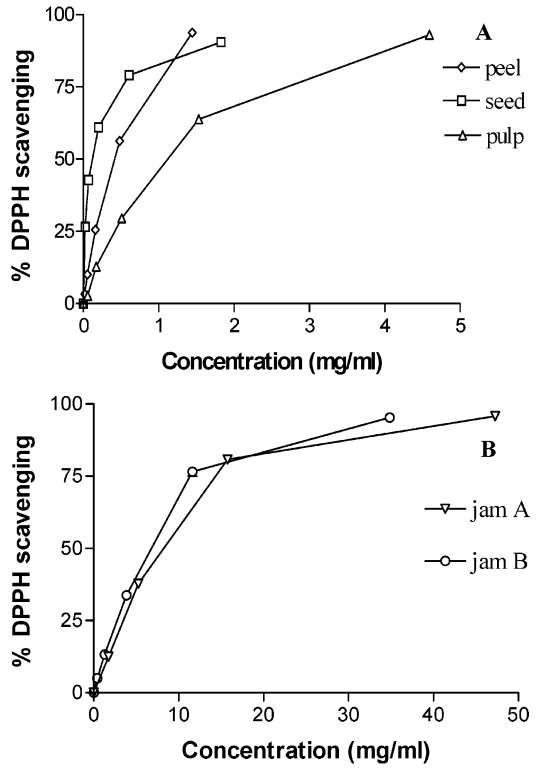


Figure 6. Antiradical activity of quince pulp, peel, and seed (**A**) and jams A and B (**B**) phenolic fractions.

total content (exponential decay; $r = 0.99793$; $p < 0.05$) and phenolics total content (exponential decay; $r = 0.99234$; $p < 0.05$). The antioxidant activity of caffeoylquinic acids can be explained by the presence of a catechol group (**Figure 2**), which confers a great stability to phenoxyl radicals by participating in electron delocalization (*24*). Additionally, the conjugated double bond in the side chain of a catechol group is likely to have a great effect in stabilizing the putative phenoxyl radical and, therefore, in enhancing antioxidant activity (*24*). Laranjinha et al. have already reported the antioxidant activity of chlorogenic acid (*24*). Any correlation between IC_{50} and flavonol glycosides total content was not found. These results are in accordance with those of Burda and Oleszek (*25*), who have done a comparison of the antioxidant activity of flavonol aglycons with the activity of its glycosides derivatives and verified that the blockage of the C-3 hydroxyl group resulted in a total loss of antioxidant activity. Glycosylation of other flavonol hydroxyls did not produce such an effect (*25*). The antioxidant activities of quercetin and kaempferol and some of its derivatives have already been reported by some authors (*25*, *26*). Probably, the seed extract had a different behavior because of its different phenolic composition. As previously referred, the seed extract had three C-glycosyl apigenin derivatives (vicenin-2, isoschaftoside, and schaftoside), one C-glycosyl luteolin derivative (lucenin-2), and three C-glycosyl chrysoeriol derivatives (stellarin-2, 6-C-pentosyl-8-C-glucoside of chrysoeriol, and 6-C-glucosyl-8-C-pentoside of chrysoeriol). As can be seen in **Figure 2**, these compounds are characterized by the presence of a hydroxyl group in position 4′ of the B ring, a 2,3-double bond in conjunction with the 4-oxo group in the C ring, and 5,7-dihydroxyl groups in the A ring. This chemical structure determines the radical scavenging effect of flavonoids (*25*, *26*). The presence of the *ortho*-dihydroxy substitution pattern in the B ring, as it happens with luteolin derivatives, is important for the antioxidant activity as well (*26*). Burda and Oleszek (*25*) and Rice-Evans et al. (*26*) have already reported the antioxidant activity of luteolin and apigenin and some of its derivatives. However, the presence of methoxyl substituent in certain positions, as occurs in chrysoeriol, can also increase the antiradical activity of flavonoids (*25*).

Concerning the organic acid extracts, the peel extract was the one that had the strongest antiradical activity (IC_{50} of 6.9 mg mL^{-1}), followed by pulp and seed extracts with very similar activities (IC_{50} of 11.6 and 12.9 mg mL^{-1}, respectively) (**Table 3** and **Figure 7A**). Jam B exhibited a stronger antioxidant activity than jam A, with IC_{50} values of 16.3 and 22.6 mg mL^{-1}, respectively (**Table 3** and **Figure 7B**). The IC_{50} values of quince pulp, peel, and jam organic acid extracts were correlated with the ascorbic acid content (exponential decay; $r = 0.99320$; $p < 0.05$) and citric acid content (exponential decay; $r = 0.98684$; $p < 0.05$). L-Ascorbic acid is a α-keto lactone with an almost planar five-membered ring (**Figure 4**). It has a double bond between the C-2 (or α) and the C-3 (or β) carbons, with the two chiral centers at positions 4 and 5 providing four stereoisomers (*27*). The acidic nature of vitamin C in aqueous solution derives from the ionization of the enolic hydroxyl on C-3, the resulting ascorbate anion being delocalized. The reversible oxidation−reduction with dehydro-L-ascorbic acid is L-ascorbic acid's most important property and the basis for its known physiological activities and stabilities (*27*). Unlike the oxidation−reduction reactions in which ascorbate donates two electrons, the antioxidant reactions use their ability to donate a single electron to free radical species (*27*). Comparing the ascorbic acid content of each organic acid fraction corresponding to IC_{50} (1.3 μg mL^{-1} for pulp and peel extracts, 0.6 and 0.9 μg

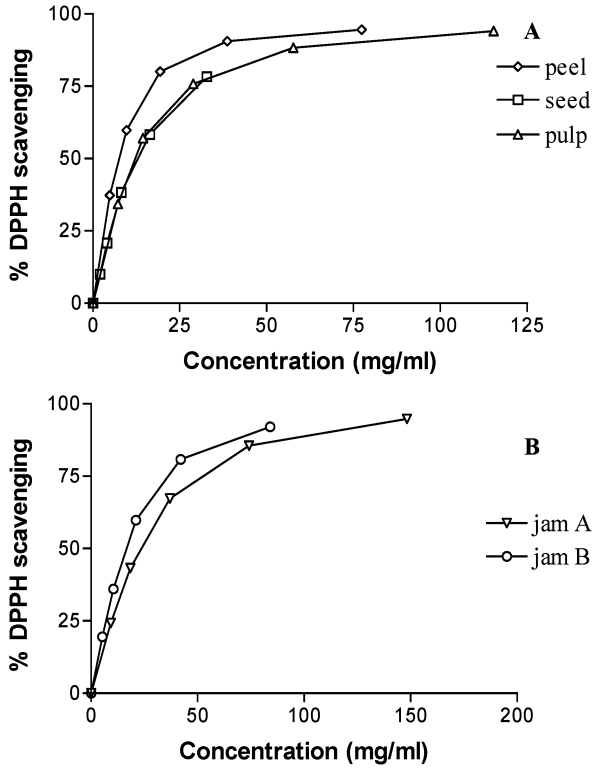


Figure 7. Antiradical activity of quince pulp, peel, and seed (**A**) jams A and B (**B**) organic acid fractions.

mL^{-1} for jams A and B extracts) with that of ascorbic acid solutions (5.6 μg mL^{-1}), it seems that vitamin C was not the only compound that contributed to antiradical activity. In fruits, citric acid protects ascorbic acid from metal-catalyzed oxidation, once it is a chelating agent (*28*). Citric acid functions as a synergist with other antioxidants (*28*). Once the seed extract exhibited great ascorbic and citric acids contents, a lower IC_{50} value was expected, which did not occur probably due to the lower malic and quinic acid contents of this extract, which results in a small organic acid total amount. The ascorbic acid content of the corresponding IC_{50} was 7.3 μg mL^{-1}, higher than 5.6 μg mL^{-1}, which may suggest the presence of compounds with prooxidant activity.

Because of the complex compositions of quince fruits and jams, interactions between different antioxidant components are likely important in terms of the overall antioxidant activity of quince fruit and jam. A comparison was made of the antiradical activity of the whole extracts (methanolic extracts) with that of its two fractions. The phenolic fraction always exhibited a stronger antioxidant activity than the whole extracts. Organic acid extracts were always the weakest in terms of antiradical activity, which seems to indicate that the phenolic fraction gives a higher contribution for the antioxidant potential of quince fruits and jams. The antioxidant activities of the analyzed samples cannot only be attributed to their phenolic and/or organic acid contents but to the result of the action of different compounds present in quince fruits and jams and to possible synergic and antagonist effects still unknown. Different amounts and types of minerals can also influence the antioxidant activity of the quince fruits and jams.

In conclusion, this study suggests that phenolic compounds are the main antioxidants in quince. This fruit and its jam can be used as good sources of antioxidants in our diet and may have relevance in the prevention of diseases in which free radicals are implicated. Additionally, quince jam byproducts (peels and seeds) are a good and cheap source of antioxidants, which could be industrially exploited.

ACKNOWLEDGMENT

We thank Branca J. Cardoso for helping with sample preparation.

LITERATURE CITED

(1) Wildman, R. E. C. Nutraceuticals: a brief review of historical and teleological aspects. *Handbook of Nutraceuticals and Functional Foods*; CRC Press: Boca Raton, Florida, 2001; pp 1−12.

(2) du Toit, R.; Volsteedt, Y.; Apostolides, Z. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. *Toxicology* **2001**, *166*, 63−69.

(3) Guthrie N.; Kurowska, E. M. N Anticancer and cholesterol-lowering activities of citrus flavonoids. *Handbook of Nutraceuticals and Functional Foods*; CRC Press: Boca Raton, Florida, 2001; pp 113−126.

(4) Andrade, P. B.; Carvalho, A. R. F.; Seabra, R. M.; Ferreira, M. A. A previous study of phenolic profiles of quince, pear, and apple purees by HPLC diode array detection for the evaluation of quince puree genuineness. *J. Agric. Food Chem.* **1998**, *46*, 968−972.

(5) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Valentão, P.; Seabra, R. M.; Ferreira, M. A. Analysis of phenolic compounds in the evaluation of commercial quince jam authenticity. *J. Agric. Food Chem.* **2000**, *48*, 2853−2857.

(6) Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A. Determination of selected phenolic compounds in quince jams by solid-phase extraction and HPLC. *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24* (18), 2861−2872.

(7) Silva, B. M.; Andrade, P. B.; Ferreres, F.; Domingues, A. L.; Seabra, R. M.; Ferreira, M. A. Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel). *J. Agric. Food Chem.* **2002**, *50*, 4615−4618.

(8) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Study of the organic acids composition of quince (*Cydonia oblonga* Miller) fruit and jam. *J. Agric. Food Chem.* **2002**, *50*, 2313−2317.

(9) Ferreres, F.; Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A. Approach to the study of C-glycosyl flavones by ion trap HPLC-PAD-ESI/MS/MS: application to seeds of quince (*Cydonia oblonga*). *Phytochem. Anal.* **2003**, *14*, 352−359.

(10) Silva, B. M.; Casal, S.; Andrade, P. B.; Seabra, R. M.; Oliveira, M. B.; Ferreira, M. A. Development and evaluation of a GC/FID method for the analysis of free amino acids in quince fruit and jam. *Anal. Sci.* **2003**, *19*, 1285−1290.

(11) Silva, B. M.; Casal, S.; Andrade, P. B.; Seabra, R. M.; Oliveira, M. B.; Ferreira, M. A. Free amino acid composition of quince (*Cydonia oblonga* Miller) fruit (pulp and peel) and jam. *J. Agric. Food Chem.* **2004**, *52*, 1201−1206.

(12) Silva, B. M.; Andrade, P. B.; Valentão, P.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Phenolic profile in the evaluation of commercial quince jellies authenticity. *Food Chem.* **2000**, *71*, 281−285.

(13) Silva, B. M.; Andrade, P. B.; Gonçalves, A. C.; Seabra, R. M.; Oliveira, M. B.; Ferreira, M. A. Influence of jam processing upon the contents of phenolics, organic acids and free amino acids in quince fruits (*Cydonia oblonga* Miller). *Eur. Food Res. Technol.* **2004**, *218*, 385−389.

(14) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant activity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.

(15) Donovan, J. L.; Meyer, A. S.; Waterhouse, A. L. Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). *J. Agric. Food Chem.* **1998**, *46*, 1247−1252.

(16) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.

(17) Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J.-P.; Pihlaja, K.; Kujala, T. S.; Heinomen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(18) Zafrilla, P.; Ferreres, F.; Tomás-Barberán, F. A. Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. *J. Agric. Food Chem.* **2001**, *49*, 3651−3655.

(19) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315−5321.

(20) Imeh, U.; Khokhar, S. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *J. Agric. Food Chem.* **2002**, *50*, 6301−6306.

(21) Shui, G.; Leong, L. P. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *J. Chromatogr. A* **2004**, *1022*, 67−75.

(22) García-Alonso, M.; Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. Evaluation of the antioxidant properties of fruits. *Food Chem.* **2004**, *84*, 13−18.

(23) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.

(24) Laranjinha, J. A.; Almeida, L. M.; Madeira, V. M. Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low-density lipoprotein peroxidation. *Biochem. Pharmacol.* **1994**, *3*, 487−494.

(25) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774−2779.

(26) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.

(27) Deshpande, S. S.; Deshpande, U. S.; Salunkhe, D. K. Human and health aspects. In *Food Antioxidants—Technological, Toxicological and Health Perspectives*; Madhavi, D. L., Desphpande, S. S., Salunku, D. K., Eds.; Marcel Dekker: New York, 1996; pp 403−412.

(28) Madhavi, D. L.; Singhal, R. S.; Kulkarni, P. R. Technological aspects of food antioxidants In *Food Antioxidants—Technological, Toxicological and Health Perspectives*; Madhavi, D. L., Desphpande, S. S., Salunku, D. K., Eds.; Marcel Dekker: New York, 1996; pp 19−224.

Received for review February 5, 2004. Revised manuscript received May 17, 2004. Accepted May 17, 2004. B.M.S. is grateful to Fundação para a Ciência e a Tecnologia for a grant (PRAXIS XXI/BD/21339/99).

JF040057V