Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity

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Abstract: The main phenolic compounds in six pear cultivars were identified and quantified using high-performance liquid chromatography/diode array detection (HPLC/DAD) and HPLC/electrospray ionisation mass spectrometry (HPLC/ESIMS). Major quantitative differences were found in the phenolic profiles. The peel contained higher concentrations of chlorogenic acid, flavonols and arbutin than the flesh, where only chlorogenic acid was detected. Total phenolics ranged from 1235 to 2005 mg kg^{-1} in the peel and from 28 to 81 mg kg^{-1} in the flesh. Ascorbic acid and dehydroascorbic acid were detected in the peel, whereas only dehydroascorbic acid was present in the flesh. The ranges of vitamin C content were from 116 to 228 mg kg^{-1} in the peel and from 28 to 53 mg kg^{-1} in the flesh. The antioxidant capacity was correlated with the content of chlorogenic acid ($r = 0.46$), while ascorbic acid made only a small contribution to the total antioxidant capacity of the fruit.

Keywords: *Pyrus communis* L; antioxidants; phenolics; ascorbic acid; dehydroascorbic acid; vitamin C; free radical scavenging activity; HPLC/ESIMS

INTRODUCTION

Various studies have suggested that diets rich in fruits and vegetables can help in the prevention of diseases such as cancer and heart attack.[1] Epidemiological studies indicate a strong inverse correlation between the consumption of fruits and vegetables and the incidence of degenerative diseases. There is considerable evidence for the role of antioxidant constituents of fruits and vegetables in the maintenance of health and the prevention of disease.[1] Phenolic compounds have the ability to prevent the oxidation of low-density lipoprotein (LDL) owing to their antioxidant properties, attributable to the free radical-scavenging properties of their constituent hydroxyl groups. The inhibition of LDL oxidation has been associated with a lower incidence of coronary diseases.[2] Among the several classes of plant phenolics, four have been reported in pear fruits: phenolic acids, flavonols, flavan-3-ols and anthocyanins.[3] Polyphenols have been widely studied in relation to their chemistry, and the changes in their content during postharvest life have been extensively reviewed.[4] Since phenolic compounds are particularly sensitive to storage factors, they can be used to indicate the physiological state of the fruit.[5] Amiot *et al*[6] observed differences in the concentration of phenolic compounds among nine pear cultivars under various storage conditions. The differences in phenolic content were determined mainly by variety rather than controlled atmosphere (CA) storage conditions. An increase in phenolics is generally considered a positive attribute to enhance the nutritional value of plant products. However, the organoleptic and nutritional characteristics of fruits and vegetables are strongly modified by the appearance of brown pigments. Browning disorders may be associated with a shift in the concentration of phenolic compounds.[7]

In the last few years there has been increasing interest in determining relevant dietary sources of antioxidant phenolics. In some countries the dietary intake of flavonoids is very low. For example, in The Netherlands the consumption of flavonoids was estimated to be 23 mg day^{-1}, whereas in the USA it was estimated to be 170 mg day^{-1}.[2]

In addition, ascorbic acid (AA), an essential vitamin present in many fruits and vegetables, plays an important role in protecting plants from oxidative stress. In humans it has been associated with the prevention of chronic diseases.[8] The concentration of AA can be influenced by various factors such

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as variety, climatic conditions, harvest practices, storage conditions and processing technologies.[9] The concentrations of AA have been reported for various fruits and vegetables.[10] Ascorbic acid acts as an antioxidant compound since it can protect fruit membranes from lipid peroxidation.[11] Pinto *et al*[12] reported a decrease in AA in pears stored under various CO_2 concentrations. That decrease was accompanied by browning of the fruit. Browning seemed to occur owing to the loss of the fruit's capacity to regenerate AA which could prevent oxidative damage. Various factors have to be considered in the evaluation of the antioxidant constituents of fruits and vegetables, such as variety, agronomic factors, maturity, harvesting methods and postharvest handling procedures.[13–16] The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay has been employed to measure the antiradical efficiency of polyphenolic compounds in grape juices and pomegranate extracts.[17]

The aim of this study was to determine the phenolic, and vitamin C (ascorbic acid and dehydroascorbic acid) contents and antioxidant capacity in six Chilean pear cultivars. Phenolic characterisation was accomplished by HPLC/DAD/MS, and browning propensity was also predicted.

EXPERIMENTAL

Reagents

Ascorbic acid (AA), citric acid, 1,2 phenylenediamine dihydrochloride (OPDA) and ethylene diamine tetra-acetic acid (EDTA) disodium salt were purchased from Sigma-Aldrich (St Louis, MO, USA). All other reagents were of analytical grade and supplied by Merck (Darmstadt, Germany). Milli-Q system ultrapure water (Millipore Corp, Milford, MA, USA) was used throughout this research.

Plant material

One yellow/green (Coscia), one red (Red D'Anjou), three green (D'Anjou, Bosc, Packams) and one green/red (Forelle) pear cultivars were compared. They were hand harvested in the southern area of Chile during January and February 2001. Damage-free pears of high quality and uniform colour and size were obtained from various packing houses. The fruits were transported to Spain by plane in refrigerated (10 °C) and immobilised packaging. After 1 day of transportation and 2 days of custar inspection, the fruits arrived at the laboratory in Murcia (Spain). Fruits free of defects and without evidence of mechanical damage were selected at commercial maturity. They were peeled and four wedges were cut vertically from each side. The flesh and peel were frozen separately in liquid nitrogen and kept at −70 °C until analysed. Sample processing and conditioning were conducted in an isolated and clean minimal processing room at 8 °C. The frozen fruits were ground into small pieces before sampling to ensure uniformity, and three replicates of 10 fruits were analysed for each cultivar.

Quality indices

Fruit weight, titratable acidity (TA), pH and soluble solids (SS) were evaluated as quality indices (Table 1). A half wedge of each replicate was liquefied in a commercial turmix blender (Moulinex, Barcelona, Spain). pH values were measured with a pH meter (Crison 501, Barcelona, Spain). TA values were determined by titrating juice samples with 0.1 M NaOH and expressed as g malic acid 100 mL^{-1}.[18] Soluble solids were measured with a hand refractometer (Atago N1, Tokyo, Japan) and expressed as g kg^{-1}. Coscia had the lowest TA, while D'Anjou, Red D'Anjou, Forelle and packams had similarly high TA values (Table 1). The pH value was highest in Coscia and lowest in Forelle pears. Differences in SS were also observed, with values ranging from 15.3 to 20.7 g 100 g^{-1} among cultivars.

Extraction of phenolic compounds

The slash and peal were frozen separately in liquid nitrogen and kept at −70 °C until analysed. The frozen fruit was ground to a fine powder with a pestle and mortar to assure uniformity. The frozen material (10 g) was homogenised in an Ultraturrax T-25 (Janke and Kunkel IKA, Labortechnik, Staufen, Germany) with 10 ml of extraction solution (methanol/formic acid, 98:2 v/v) for 1 min on ice. The homogenate was filtered through filter cloth, centrifuged at 11340 × *g* for 3 min and passed through a 0.45 μm polyether sulfone filter (Millipore Corp., Bedford, USA). The extracts were used for HPLC analysis and free radical scavenging assay.

Table 1. Quality indices of pear cultivars[a]

Cultivars	Date of harvest	Weight (g)[b]	Titratable acidity (g malic acid 100 mL^{-1} juice)	pH	Soluble solids (g 100 g^{-1})
D'Anjou	09/02/01	174.0 (13.9)	0.20 (0.03)	4.42 (0.06)	176 (4)
Red D'Anjou	17/02/01	177.1 (22.6)	0.21 (0.02)	4.47 (0.08)	192 (11)
Bosc	22/02/01	211.9 (17.8)	0.15 (0.02)	4.58 (0.05)	207 (4)
Forelle	26/02/01	175.1 (13.5)	0.23 (0.03)	4.31 (0.02)	198 (2)
Coscia	18/01/01	94.1 (5.1)	0.06 (0.00)	5.26 (0.08)	153 (2)
Packams	23/02/01	235.2 (13.9)	0.23 (0.03)	4.39 (0.13)	167 (9)

[a] Standard deviations ($n = 3$) in parentheses.
[b] Data are the mean of 10 replicates.

HPLC/DAD/MS analysis

Chromatographic separation was carried out on a reverse phase C_{18} LiChroCART column (25 cm × 0.4 cm, particle size 5 μm, Merck) with water/acetic acid (95:5 v/v) (A) and methanol (B) as the mobile phases, using a gradient starting with 10B:90A and reaching 15B:85A after 30 min and 50B:50A after 62 min. The flow rate was 1.0 ml min^{-1} and the injection volume was 80 μl for quantitative analysis and 20 μl for qualitative analysis. The HPLC system, equipped with a diode array detector (DAD) and a mass detector in series, consisted of an HPLC binary pump (G1312A), an autosampler (G1313A), a degasser (G1322A) and a photodiode array detector (G1315B) controlled by software (v A08.03) from Agilent Technologies (Waldbronn, Germany). The mass detector was an ion trap mass spectrometer (G2445A, Agilent Technologies) equipped with an electrospray ionisation (ESI) system and controlled by software (v 4.0.25). The heated capillary and the voltage were maintained at 350 °C and 4 kV respectively. The full-scan mass spectra of the phenolic compounds were measured from *m/z* 100 to 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionisation mode for flavonols, hydroxycinnamic acids and flavan-3-ols and in the positive ionisation mode for anthocyanins. Total ion chromatograms (TICs) were determined for two alternating scan events: (1) MS (full scan) was used to measure the pseudomolecular ions ($[M-H]^-$), giving the molecular masses of the components, and (2) MS/MS was used to break down the most abundant pseudomolecular ions from MS (full scan). UV chromatograms were recorded at 280, 330, 350 and 520 nm although the chromatogram at 350 nm was selected for Figure 1. Retention times, UV-vis spectra and full MS and MS/MS scans were compared with those of a number of commercially available authentic aglycones and glycosylated flavonols and anthocyanins. Individual flavonols were quantified by comparison with external standards. For flavonols, quercetin 3-*O*-glucoside was isolated in our laboratory from onion, quercetin 3-*O*-rutinoside was purchased from Merck and quercetin 3-*O*-galactoside was obtained from Extrasynthese (Genay, France). Hydroxycinnamic acid derivatives were quantified as chlorogenic acid (Sigma-Aldrich, St Louis, MO, USA). For anthocyanins, cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-arabinoside and cyanidin 3-*O*-rutinoside were obtained from Polyphenols Lab (Sandnes, Norway). The concentrations were expressed as mg 100 g^{-1} fresh weight.

Extraction and analysis of vitamin C

Ascorbic acid (AA) and dehydroascorbic acid (DHA) contents were determined as described by Zapata and Dufour.[19] Pear flesh or peel (10 g) was homogenised in 10 ml of methanol/water (5:95 v/v) plus citric acid and EDTA (0.5 g l^{-1}) in an Ultraturrax T-25 (Jauke and Kunkel IKA, dabortechnik, Staufen, Germany) for 3 min. Then the pH was adjusted to 2.2–2.4 and the extract was adsorbed onto a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA, USA). The resultant solution was derivatised with 1,2-phenylenediamine dihydrochloride (OPDA) (Fluka Chemika, Neu-Ulm, Switzerland) for 37 min before analysis by HPLC. AA and DHA contents were expressed as mg 100 g^{-1} fresh weight. Three replicate analyses of 10 fruits were performed on the flesh and peel. Standard solutions, column conditioning and derivatisation procedures were as previously described.[20] Ascorbate determinations were carried out in triplicate on the peel and flesh.

Antioxidant capacity

The analysis was based on the evaluation of the free radical-scavenging capacity of the extracts according to the technique reported by Brand-Williams *et al*.[21] The assay used a commercially available free radical ($DPPH^{\bullet+}$, 2,2 diphenyl-1-picrylhydrazyl) which is soluble in methanol, and the antioxidant capacity was evaluated after 20 min of reaction at 20 °C by measuring the absorbance at 515 nm in a spectrophotometer (Shimadzu UV-1603, Tokyo, Japan). Standard solutions of 5.7 mM L-ascorbic acid (Aldrich, Steinheim, Germany) in water were prepared. Diluted standards or diluted extract samples were used on the day of preparation, except for ascorbic acid solutions which were used within 1 h of preparation. The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC).[17]

RESULTS AND DISCUSSION

HPLC/DAD and HPLC/ESIMS analysis of pear phenolics

The compounds described in this subsection were found in the peel of pears but not in the flesh. Twelve flavonoid peaks were detected by HPLC/DAD and showed almost identical UV spectral profiles (maxima at 257–259 and 353–355 nm) (Fig 1). Only peak **6** showed slightly different UV maxima (Table 2). Pseudomolecular ions $[M-H]^-$ at *m/z* 463 were found for the peaks at 45.7 and 46.9 min (**1** and **3** respectively). Fragmentation of these ions provided a characteristic *m/z* at 301 (quercetin aglycone residue). They were identified as quercetin 3-*O*-galactoside (**1**) and quercetin 3-*O*-glucoside (**3**) by co-chromatography with authentic markers, in agreement with previous studies[22–24] (Table 2). Two peaks with the same $[M-H]^-$ at *m/z* 609 (peaks **2** and **4**) and main fragment ions at *m/z* 301 and 463 were found (Table 2 and Fig 1). Peaks **2** and **3** were not properly resolved in the UV chromatogram but were distinguishable if the single ion chromatograms were plotted (Fig 1). Peak **4** was identified as quercetin 3-*O*-rutinoside by comparison with the authentic

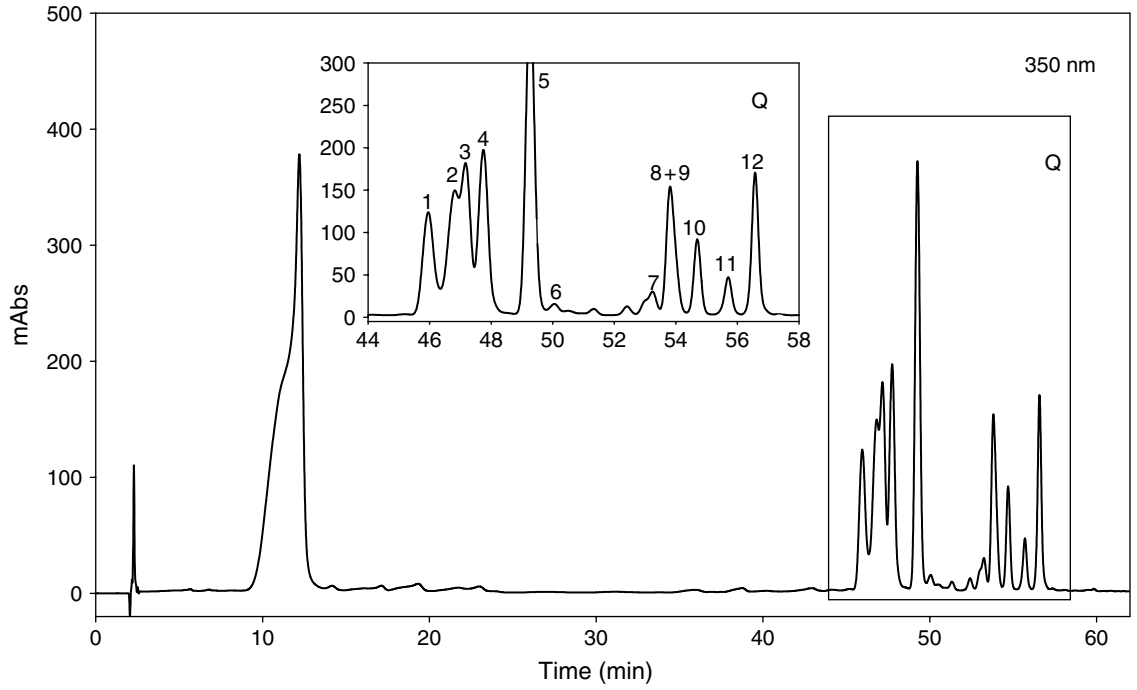

Figure 1. HPLC chromatogram of pear peel at 350 nm. See Table 2 for flavonol identification (Q).

Table 2. HPLC/DAD and HPLC/ESIMS of flavonols in peel of pears

Peak	*Rt* HPLC (min)	HPLC/UV/DAD (nm)	$[M-H]^-$ in MS (*m/z*)	$[M-H]^-$ in MS/MS (*m/z*)	Structure assignment
1	45.7	257,353	463	301	Q 3-*O*-galactoside
2	46.5	257,353	609	463, 301	Q rhamnose-hexoside
3	46.9	257,353	463	301	Q 3-*O*-glucoside
4	47.5	257,355	609	463, 301	Q 3-*O*-rutinoside
5	49.0	257,355	549	505, 463, 301, 301	Q hexose-malonate
6	49.8	265,362	682	638, 506, 464, 446, 300	Q pentose-hexose-malonate
7	53.0	257,355	477	315, 314	I hexoside
8	53.6	257,355	623	478, 460, 315, 315	I rhamnose-hexoside
9	53.9	257,355	477	314, 315	I hexoside
10	54.5	257,355	623	478, 315	I rhamnose-hexoside
11	55.5	259,355	563	520, 477, 315	I hexose-malonate
12	56.4	257,355	563	519, 477, 315	I hexose-malonate
Std	45.6	257,355	463	302	Q 3-*O*-galactoside
Std	46.9	257,353	463	301	Q 3-*O*-glucoside
Std	47.5	259,355	609	301, 300	Q 3-*O*-rutinoside

Q, quercetin; I, isorhamnetin; Std, standard: *RT*, retention time.

marker.[23,24] Peak **2** was tentatively identified as quercetin 3-*O*-rhamnosylgalactoside.[23,24] Peak **5** (*m/z* 549) was identified as an acylated flavonoid after mineral acid incubation. Dicarboxylic acid derivatives of flavonoids are especially sensitive to decomposition by mineral acids, releasing the acyl residue at room temperature after a few minutes. MS/MS of the *m/z* 549 peak produced three ions at *m/z* 505, 463 and 301 (Table 2). The fragment at *m/z* 505 came from *m/z* 549 by the loss of CO_2, confirming the acylation of this flavonol. The fragment at *m/z* 463 (M – malonyl) indicated that peak **5** was a malonated quercetin glucoside, as previously described in some pear cultivars.[23,25] Peak **6** produced an $[M-H]^-$ at *m/z* 682, which after MS/MS was fragmented into three major ions (*m/z* 638, 506 and 300) and two minor ones (*m/z* 464 and 446) (Table 2 and Fig 1). The ion at *m/z* 638 was formed as a result of the loss of a carboxyl residue from the molecular ion. The fragments at *m/z* 464 and 446 suggested hexose and pentose glycosylation respectively, and their co-occurrence indicated substitution at different positions of the aglycone ring. The fragment at *m/z* 506 coincided with quercetin malonylglucoside minus CO_2, suggesting that the malonyl residue could be linked to the glucose residue. As peak **5** was described as a malonated quercetin 3-*O*-glucose, the at *m/z* 506 fragment could be tentatively identified as a quercetin glycosylated with a hexose-malonate in position 3 and a pentose in another position

Table 3. HPLC/DAD and HPLC/ESIMS of anthocyanins in red peel of pears

Peak	*Rt* HPLC (min)	HPLC/UV/DAD (nm)	$[M-H]^+$ in MS (*m/z*)	$[M-H]^+$ in MS/MS (*m/z*)	Structure assignment
13	35.9	277,517	449	287	Cy 3-*O*-galactoside
14	39.7	283,517	449	287	Cy 3-*O*-glucoside
15	40.8	281,515	419	287	Cy pentoside
16	41.9	281,517	419	287	Cy 3-*O*-arabinoside
17	42.8	279,517	595	449, 287	Cy 3-*O*-rutinoside
Std	35.8	279,519	449	287	Cy 3-*O*-galactoside
Std	39.4	281,519	449	287	Cy 3-*O*-glucoside
Std	41.8	281,517	419	287	Cy 3-*O*-arabinoside
Std	42.9	281,519	595	449, 287	Cy 3-*O*-rutinoside

Cy, cyanidin; Std, standard; *Rt*, retention time.

(probably 7) of the flavonol ring (Table 2). According to the literature, acylation of pear flavonoids takes place at C-6″.[25] In the present study, quercetin malylglucoside was not detected in any of the samples analysed, although it was previously described in Guyot pears.[23]

Peaks **7**–**12** showed isorhamnetin fragment ions at *m/z* 315 in the MS/MS analyses (Table 2 and Fig 1). Peaks **7** and **9** yielded the same $[M-H]^-$ at *m/z* 477, which could be tentatively identified as isorhamnetin 3-*O*-galactoside and isorhamnetin 3-*O*-glucoside respectively (Table 2).[23,24] Peaks **8** and **9** were easily separated using single ion chromatography in the HPLC/MS analyses (Fig 1). Peaks **8** and **10** showed molecular ions at *m/z* 623 and minor fragment peaks at *m/z* 478 ($[M-H-\text{deoxyhexose}]^-$), 460 ($[M-H-\text{hexose}-H_2O]^-$; peak **8** only) and 315. They were tentatively identified as isorhamnetin 3-*O*-rhamnosylgalactoside and isorhamnetin 3-*O*-rhamnosylglucoside respectively according to previous results.[23,24] Peaks **11** and **12** were found as isorhamnetin glycosides acylated with dicarboxylic acids (Fig 1). The *m/z* value for their pseudomolecular ions was 563 for both compounds, and MS/MS experiments yielded major peaks at *m/z* 520 ($[M-H-CO_2]^-$) and 477 ($[M-H-\text{malonic acid}]^-$) (Table 2). They tentatively corresponded to isorhamnetin 3-*O*-galactoside-malonate (**11**) and isorhamnetin 3-*O*-glucoside-malonate (**12**) (Table 2 and Fig 1).[23,25]

Among the six pear cultivars analysed, only Coscia showed all the flavonols found in this study (Table 2 and Fig 1). In addition, this cultivar was the only one that provided a new flavonol (peak **6**). Packams contained nine flavonols, including quercetin 3-*O*-malonylglucoside.[25] Quercetin 3-*O*-galactoside was not detected in D'Anjou, although Spanos and Wrolstad[22] found it in the juice of this cultivar.

Five anthocyanin peaks were detected by HPLC/DAD analyses of red peel extracts (maxima at 277–283 and 515–517 nm) (Table 3). The data revealed an *O*-glycosylation in position 3 of the main ring of the anthocyanin. Peel anthocyanins showed a higher response factor in the ion trap mass spectrometer in the positive mode[26] than in the negative one.[27] Peaks **13** and **14** showed identical pseudomolecular ions at *m/z* 449, and their fragmentation gave a major peak at *m/z* 287 in both cases (Table 3). Single-mass analyses of the authentic markers cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside followed by addition to the pear samples confirmed the occurrence of these anthocyanins. These anthocyanins had been previously reported in Red Delicious pears.[28] Peaks **15** and **16** showed ions at *m/z* 419, with the same major cyanidin fragment ions at *m/z* 287 ($[M+H-\text{pentose}]^+$) (Table 3). Peak **16** was identified as cyanidin 3-*O*-arabinoside by comparison with the authentic marker.[28,29] The other cyanidin pentoside with a shorter retention time than cyanidin 3-*O*-arabinoside was tentatively identified as cyanidin 3-*O*-xyloside.[28] Peak **17** showed an $[M+H]^+$ at *m/z* 595, which gave MS/MS fragments was at *m/z* 287 and 449 ($[M+H-\text{deoxyhexose}]^+$), identified as cyanidin 3-*O*-rhamnosylhexoside[30] (Table 3). All five anthocyanins were detected in the peel of Red D'Anjou, while only cyanidin 3-*O*-galactoside, cyanidin 3-*O*-arabinoside and cyanidin 3-*O*-rutinoside were found in Forelle. Anthocyanin acyl derivatives were not detected in the peel of red pears as had been reported in Red Delicious.[28]

Phenolic content in pear peel and flesh

Chlorogenic acid was detected as the major hydroxycinnamic acid derivative. Its content was always higher in the peel than in the flesh (Table 4).[3] The levels of hydroxycinnamic compounds found were similar to previously reported values.[16,31] In addition, several other hydroxycinnamates were detected by their characteristic UV spectra. In the peel the highest hydroxycinnamic acid concentrations were found in Forelle and Red D'Anjou (Table 4). In the flesh, however, the highest hydroxycinnamic acid contents were found in D'Anjou and Red D'Anjou, followed by Packams Bosc, Forelle and Coscia (Table 4). Enzymatic browning of pears has been associated with the presence of chlorogenic acid in the fruit, although the extent of browning seems to be mostly dependent on the level of maturity.[6]

Epicatechin was the major flavan-3-ol compound found in the peel. Its concentration was highest in Packams, followed by Bosc, while Forelle showed only half this content (Table 4). Epicatechin was detected

Table 4. Contents of hydroxycinnamic acid derivatives, flavan-3-ols, flavonols, anthocyanidins, total phenolics and arbutin (mg 100 g^{-1} fresh weight) in peel and flesh of pear cultivars[a]

	Cultivar					
Compounds	D'Anjou	Red D'Anjou	Bosc	Forelle	Coscia	Packams
Peel						
Hydroxycinnamics	24.2 (2.1)	37.3 (1.7)	19.3 (1.4)	38.2 (3.3)	22.3 (0.2)	14.2 (5.2)
Flavan-3-ols	1.4 (0.1)	3.1 (0.1)	21.1 (1.7)	11.6 (0.8)	3.3 (0.9)	22.4 (10.0)
Flavonols	16.2 (3.0)	54.7 (1.7)	9.5 (1.6)	55.9 (1.8)	40.6 (4.9)	30.5 (8.2)
Anthocyanidins	ND	12.0 (0.2)	ND	1.2	ND	ND
Arbutin	81.7 (3.3)	105.5 (8.6)	115.8 (2.2)	58.3 (4.8)	61.1 (3.2)	72.7 (10.6)
Total phenolics	123.5 (2.6)	200.5 (5.6)	166.5 (2.2)	164.1 (5.2)	127.3 (5.2)	139.8 (14.1)
Flesh						
Hydroxycinnamics	8.1 (0.8)	7.9 (1.1)	3.5 (0.4)	3.4 (0.4)	2.8 (0.4)	4.2 (0.5)

[a] Standard deviations ($n = 3$) in parentheses.
ND, not detected.

Table 5. Flavonol content (mg 100 g^{-1} fresh weight) in peel of pear cultivars[a]

		Cultivar					
Peak	Flavonol	D'Anjou	Red D'Anjou	Bosc	Forelle	Coscia	Packams
1	Q3Gal	ND	3.0 (4)	0.5 (0.1)	3.2 (0.2)	4.2 (1.1)	1.9 (0.7)
2 + 3	QRH + Q3Glc	tr	6.4 (7)	1.6 (0.4)	3.2 (0.1)	9.6 (0.6)	4.8 (1.2)
4	Q3Rut	5.5 (0.5)	14.2 (8)	0.3 (0.1)	1.1 (0.1)	5.9 (0.4)	2.0 (0.4)
5	QHM	2.4 (0.6)	6.6 (2)	0.8 (0.4)	3.2 (0.1)	10.0 (1.8)	ND
6	QPHM	ND	ND	ND	ND	0.1 (0.0)	ND
7	IH (1)	ND	1.4 (1)	0.6 (0.1)	4.0 (0.1)	0.6 (0.2)	1.9 (0.9)
8 + 9	IRH (1) + IH (2)	1.9 (0.4)	10.7 (3)	2.6 (0.3)	13.0 (0.5)	4.2 (0.3)	10.3 (3.2)
10	IRH (2)	3.2 (0.4)	6.0 (3)	0.8 (0.1)	5.2 (0.3)	1.8 (0.1)	8.8 (1.4)
11	IHM (1)	ND	1.1 (1)	0.3 (0.1)	2.8 (0.1)	0.8 (0.1)	ND
12	IHM (2)	2.5 (0.5)	5.4 (2)	1.9 (0.3)	16.1 (0.7)	3.5 (0.4)	0.7 (0.3)

[a] Standard deviations ($n = 3$) in parentheses.
Q3Gal, quercetin 3-*O*-galactoside; QRH, quercetin rhamnose-hexoside; Q3Glc, quercetin 3-*O*-glucoside; Q3Rut, quercetin 3-*O*-rutinoside; QHM, quercetin hexose-malonate; QPHM, quercetin pentose-hexose-malonate; IH (1), isorhamnetin hexoside; IRH (1) isorhamnetin rhamnose-hexoside; IH (2), isorhamnetin hexoside; IRH (2), isorhamnetin rhamnose-hexoside; IHM (1), isorhamnetin hexose-malonate; IHM (2), isorhamnetin hexose-malonate; ND, not detected; tr, trace.

only in small amounts in Coscia, Red D'Anjou and D'Anjou. The flavan-3-ol contents found in this study were slightly higher than those reported by Macheix *et al*[3] but were in agreement with the values of Escarpa and González.[16] Epicatechin has also been reported in the peel of other pear cultivars.[31] However, the variation in its concentration seems to have no significance in relation to browning, as is the case with flavan-3-ols such as catechin.[32]

Flavonols were located in the peel but not in the flesh. The variability in the content of these compounds was very high. Forelle and Red D'Anjou had the highest flavonol contents (55.9 and 54.7 mg k g^{-1} peel respectively). Coscia and Packams had intermediate flavonol contents, whereas the levels were very low in D'Anjou and Bosc pears (Table 4). The quantification of individual flavonols in the various cultivars is shown in Table 5. Coscia had the highest level of quercetin derivatives, while Forelle had the highest content of isorhamnetin derivatives (Table 5). The flavonol contents in the cultivars studied were lower than those reported in Blanquilla and Decana pears.[16]

Table 6. Anthocyanin content (mg 100 g^{-1} fresh weight) in peel of red pear cultivars[a]

		Cultivar	
Peak	Anthocyanin	Forelle	Red D'Anjou
13	Cy3Gal	1.02 (0.11)	11.40 (0.13)
14	Cy3Glc	ND	0.03 (0.00)
15	CyP	ND	0.02 (0.00)
16	Cy3Arab	0.01 (0.00)	0.10 (0.02)
17	Cy3Rut	0.2 (0.01)	0.55 (0.06)

[a] Standard deviations ($n = 3$) in parentheses.
Cy3Gal, cyanidin 3-*O*-galactoside; Cy3Glc, cyanidin 3-*O*-glucoside; CyP, cyanidin pentoside; Cy3Arab, cyanidin 3-*O*-arabinoside; Cy3Rut, cyanidin 3-*O*-rutinoside; ND, not detected.

Anthocyanin pigments were found only in the peel of Red D'Anjou and Forelle. Red D'Anjou had the highest pigment content (12.0 mg 100 g^{-1} peel), while Forelle contained a much smaller amount (1.2 mg 100 g^{-1} peel) (Table 4). Cyanidin 3-*O*-galactoside was the main pigment, followed by cyanidin 3-*O*-rutinoside (Table 6).

Table 7. Ascorbic acid (AA), dehydroascorbic acid (DHA) and antioxidant capacity by DPPH assay (ascorbic acid equivalent antioxidant capacity, AEAC) in peel and flesh of pear cultivars[a]

	Peel			Flesh		
Cultivar	AA	DHA	AEAC	AA	DHA	AEAC
D'Anjou	3.9 (1.0)	12.7 (1.9)	120.2 (10.5)	3.0 (0.5)	ND	23.1 (4.8)
Red D'Anjou	5.3 (1.2)	15.4 (2.6)	124.0 (6.5)	4.4 (1.1)	ND	19.5 (11.1)
Bosc	2.6 (0.2)	14.3 (1.7)	108.2 (9.9)	2.8 (0.9)	ND	35.2 (7.6)
Forelle	4.4 (1.6)	18.4 (3.5)	113.1 (8.2)	3.0 (0.8)	2.3 (1.4)	30.3 (11.3)
Coscia	3.6 (0.9)	8.0 (0.4)	75.4 (13.7)	4.4 (0.4)	ND	9.9 (1.5)
Packams	4.1 (0.9)	15.5 (2.2)	98.7 (14.1)	3.8 (0.6)	1.0 (0.8)	26.1 (5.4)

[a] Means in mg 100 g^{-1}. Standard deviations ($n = 3$) in parentheses.
ND, not detected.

Arbutin (hydroquinone 1-β-D-glucoside) was found only in the peel (Table 4). Bosc and Red D'Anjou had the highest arbutin contents (1158 and 1055 mg kg^{-1} peel respectively). Arbutin has been found in the peel, stem, core and cortex as well as in the flesh of pears.[16,33] The highest levels have been detected particularly in immature fruits. Its possible role as a polyphenol oxidase substrate could not be established.[34] In our study the arbutin contents were higher than those previously reported in D'Anjou and Bosc pear flesh.[5]

Regarding total phenolics in the peel, Red D'Anjou had the highest content (200.5 mg kg^{-1} peel), followed by Bosc and Forelle (166.5 and 164.1 mg 100 g^{-1} peel respectively). D'Anjou, Coscia and Packams had the lowest phenolic contents (123.5, 127.3 and 139.8 mg 100 g^{-1} peel respectively) (Table 4).

With regard to phenolics in the flesh, only chlorogenic acid was present in sufficient amounts to allow its quantification (Table 4). The cultivars studied showed similar profiles for phenolic compounds in the peel and the flesh, with differences in their contents being attributable to the cultural type.

Ascorbic acid, dehydroascorbic acid and antioxidant capacity in pear tissue

Large differences were found in the contents of AA and DHA between the flesh and the peel. Both AA and DHA were detected in the peel, although the content of DHA was threefold higher than that of AA in all cultivars (Table 7). The DHA content in the flesh was very low, and it was only detected in Forelle and Packams pears. On the other hand, similar AA contents were found in both the peel and the flesh (Table 7). When the cultivars were compared, Forelle showed the highest vitamin C (AA + DHA) content in both the flesh and the peel. This finding could be related to the browning susceptibility of some cultivars and the capacity to avoid browning during postharvest handling and storage. Previous studies have shown higher AA contents than these found in the present work (7.2 mg 100 g^{-1} for Conference pears and 6.1–8.2 mg 100 g^{-1} for Rocha pears).[35] Veltman *et al*[35] suggested that browning is initiated in a cultivar-dependent manner when a certain AA level threshold is passed. They explain that the threshold value depends on the cultivar, picking date and growing location.

The antioxidant capacity of the pear extracts was located mainly in the peel (Table 7). The peel of the red pears generally showed higher antioxidant capacities than that of the yellow cultivar (Coscia). The flesh extracts showed large differences in antioxidant capacity, with Coscia having the lowest antioxidant capacity among the cultivars studied (Table 7). The chlorogenic acid content of the peel and the antioxidant capacity were positively correlated ($r = 0.46$), while a lower correlation was found with the flavonol and arbutin contents. The observed antioxidant capacity could not be well explained by the contents of individual phenolics. In addition, a negative correlation between vitamin C content and antioxidant capacity was observed.

The antioxidants supplied by these pear cultivars in the diet were determined on a typical serving basis (Table 8). A pear serving of 100 g of edible fruit was considered to consist of 82 g of flesh and 18 g of peel. Different levels of dietary intake of flavonoids have been estimated. One recent study reported the intake to be 55.2 mg day^{-1}.[36] The amount of phenolics in one serving of pears varied between 27.2 and 40.7 mg, depending on the cultivar (Table 8). For adults the dietary needs of vitamin C are met by a minimum intake of 60 mg day^{-1}, although the recommended dietary allowance is 75–90 mg day^{-1}.[8] The vitamin C content in the pear cultivars studied ranged between 5.5 and 8.4 mg per serving (Table 8). Although the contribution of pears to dietary intake of vitamin

Table 8. Total phenols, vitamin C and antioxidant capacity by DPPH assay (ascorbic acid equivalent antioxidant capacity, AEAC) in mg per serving of pear cultivars[a]

Cultivar	Total phenols	Vitamin C	AEAC
D'Anjou	28.9 (1.2)	5.5 (0.8)	40.8 (7.8)
Red D'Anjou	40.7 (1.2)	7.2 (0.6)	37.7 (8.6)
Bosc	32.8 (0.4)	5.4 (0.5)	48.6 (4.6)
Forelle	32.3 (1.3)	8.4 (1.4)	45.5 (11.2)
Coscia	30.2 (1.4)	6.0 (0.2)	24.3 (2.8)
Packams	27.2 (2.8)	7.2 (1.2)	38.1 (3.9)

[a] Serving = 100 g of edible pear (82 g flesh + 18 g peel). Standard deviations ($n = 3$) in parentheses.

C is very low in contrast with other fruits such as oranges,[37] the general recommendation is to eat a wide variety of fruits and vegetables which can supplement each other in terms of dietary requirements. Among various fruits and vegetables, pears were classified in the group with low antioxidant capacity by Prior and Cao.[38] When the antioxidant capacities per serving were compared, the cultivars studied showed similar values, except for Coscia which had a relatively low antioxidant capacity. Higher antioxidant activities have been reported in pigmented fruits such as prunes, berries, pomegranates and in plums.[39,40] In these fruits the antioxidant capacity is correlated mainly with the presence of anthocyanins and other phenolics. The contribution of phenolic compounds to the antioxidant capacity in pears was much greater than that of vitamin C.

CONCLUSIONS

From the nutritional point of view, since most of the phenolics were located in the peel, the consumption of unpeeled pears is recommended to maximise the dietary intake of antioxidant compounds. In addition, a general recommendation is to select red and green pears because of their higher levels of antioxidant components (phenolics, vitamin C and antioxidant capacity). For postharvest suitability, cultivar selection is also an important parameter. Since development of browning and susceptibility to bruising are among the factors which limit the storage life of pears, cultivars with high levels of antioxidant components are recommended.

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