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ScienceDirect
Food Chemistry 104 (2007) 518–527
Food Chemistry
www.elsevier.com/locate/foodchem

Procyanidins from pine bark: Relationships between structure, composition and antiradical activity

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Received 16 June 2006; received in revised form 5 October 2006; accepted 28 November 2006

Abstract

Barks of *Pinus pinaster* and *Pinus radiata* were studied as source of procyanidins; these raw materials, considered a byproduct of forestal industry, were extracted with ethanol. The extract was partially fractionated to obtain an aqueous fraction (FA) containing a great part of the procyanidins from barks and with potential application to both food and medical fields. FAs were rich in polyphenols, 65–87% of which were procyanidins; the mean degree of polymerization (mDP) was 7.9 for radiata (rFA) and 10.6 for pinaster (pFA) varieties.

The aqueous fractions were chromatographed on Sephadex LH-20 to obtain specific fractions differing in DP and composition. These fractions were analysed by thiolysis with cysteamine, followed by RP-HPLC. Results showed that (+)-catechin was the main terminal unit for both barks and also the main extension unit for radiata. In contrast (−)-epicatechin was predominant as extension unit in pinaster and this could have implications for applications in oils, emulsions and biological systems.

In terms of antiradical activity, expressed as specific antiradical units, the entire rFA gave the best results, together with rF5. For *P. pinaster* bark, the best results were achieved for fractions F5–F8, with DP 7–22. The whole fraction from radiata represents an economic alternative of great interest because the fractionation costs can be avoided.

Keywords: *Pinus pinaster*; *Pinus radiata*; Bark; Antiradical power; Procyanidins; Aqueous fraction; Fractionation

1. Introduction

Great interest is currently centred on potential benefits of plant polyphenols as complements to the organism's antioxidant defence system. Polyphenols are potent free radical-scavengers and preventive against cardiovascular diseases, cancer and other disorders; they are increasingly used as natural food additives, acting as flavouring, colouring and antioxidant agents (Plumb, de Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998). Among the different phenolics, flavonoids, phenolic acids, stilbenes and tannins, especially condensed tannins (proanthocyanidins), are particularly important (Hagerman et al., 1998). Proanthocyanidins are the most abundant polyphenols in plants after lignins, and they may represent up to 50% in several barks (Matthews et al., 1997); they can be divided into procyanidins and prodelphynidins. Pycnogenol, basically a procyanidin mixture from maritime pine, is probably the most studied phenolic from trees.

Procyanidins are composed of oligomers and polymers, consisting of (+)-catechin and/or (−)-epicatechin units linked mainly through C4 → C8 and/or C4 → C6 bonds (B-type). These flavan-3-ol units can be doubly linked by a C4 → C8 bond and an additional ether bond from O7 → C2 (A-type). Most of the activities of procyanidins, including the free radical-scavenging capacity, largely depend on their structure, particularly their degree of polymerization (DP) (Gaulejac, Vivas, Freitas, & Bourgeois, 1999; Touriño et al., 2005). Moreover, the presence of gallate esters seems decisive for the regulation of the cell cycle

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doi:10.1016/j.foodchem.2006.11.071

by catechins (Liberto & Cobrinik, 2000). In food systems, partition phenomena of procyanidins play an important role in the preservant action (Frankel, 2001). The main difficulty in studies on procyanidins is probably that of obtaining them in an individual molecular form. The complete purification of a procyanidin with a DP above five is almost impossible. Therefore, for studying their structures and properties, mixtures more or less polymerized are often employed (Guyot, Marnet, & Drilleau, 2001). Moreover, synergistic effects of active mixtures make plant extracts and fractions more interesting than pure compounds for functional food applications.

Many methods have been proposed for separating procyanidins according to their DP. For analytical purposes, a suitable separation can be achieved by normal-phase HPLC, TLC or reversed-phase HPLC. On a preparative scale, gel chromatography with different packings, such as Sephadex G-25, LH-20 and Toyopearl TSK HW 40, has been used (Kantz & Singleton, 1990; Karonen, Loponen, Ossipov, & Pihlaja, 2004). Normal-phase HPLC can separate procyanidins according to their degree of polymerization up to decamers, but this method is specific for monomers and oligomers and results in a severe underestimation of procyanidins, considering the prevalence of the polymers in nature (Czochanska, Foo, Newman, & Porter, 1980). Reversed-phase HPLC is the method commonly used for the separation of flavan-3-ol monomers and some small oligomers, especially dimers and trimers. However, there is difficulty in determining the DP using reversed-phase methods since oligomers elute in non-sequential order. Furthermore, analysis of higher oligomeric procyanidins is not feasible, since the number of isomers increases with increasing degrees of polymerization. This effect results in a retention time overlap of isomers of differing DP, causing the higher oligomers to co-elute as a large unresolved peak (Santos-Buelga & Williamson, 2003). The technique of thin-layer chromatography (TLC), with a silica phase, permits the separation of oligomeric proanthocyanidins up to heptamers (Lea, 1978). This method can be used only for qualitative analysis. Sephadex LH-20 and Toyopearl TSK HW-40 or TSK HW-50 columns are classically used to fractionate proanthocyanidins on the basis of molecular size; these are eluted with a mobile phase of alcohol–water or acetone–water. Large molecular weight procyanidins are then recovered with 60–70% acetone in water, with no further separation (Taylor, Barofsky, Kennedy, & Deinzer, 2003; Touriño et al., 2005).

Depolymerization by thiolysis has been proved as an efficient method for determining the nature of the flavan-3-ol units within procyanidins and for determining the average degree of polymerization. Thiolysis is performed in the presence of acid and a nucleophilic reagent, such as toluene-α-thiol (Rigaud, Perez-Ilzarbe, Da Silva, & Cheynier, 1991), cysteamine hydrochloride (Torres & Selga, 2003) or phloroglucinol (Kennedy & Jones, 2001). The extension subunits of procyanidins are attacked by the nucleophilic reagent to form the corresponding thio-derivatives and the terminal unit is released as the free flavan-3-ol. Degradation products can be analysed by reversed-phase HPLC, and the results will provide information on the nature of the extension and terminal units and on the average degree of polymerization.

Pine is one of the plants with the highest content of procyanidins. The phenolics of pine bark are (+)-catechin, (−)-epicatechin, dihydroquercetin, phenolic acids and, most of all procyanidin dimers, trimers, oligomers and polymers (Wood, Senthilmohan, & Peskin, 2002). Pine bark procyanidins have diverse biomedical applications (Packer, Rimbach, & Virgili, 1999; Rohdewald, 2002). Several constituents of pine bark extracts, such as gallic, protocatechuic acids and catechin, are readily adsorbed by human skin and make the preparations useful for topical application (Sarikaki et al., 2004). Besides, because pine procyanidins are devoid of gallate esters, which appear to interfere with crucial cell functions, they may be innocuous chemopreventive agents of choice for many applications (Touriño et al., 2005). The use of polyphenols as food antioxidants is also frequent, especially in fatty foods, such as fish, making it possible to prolong the storage time of several species both fresh and frozen products (Pazos, Alonso, Fernández-Bolaños, Torres, & Medina, 2006). Another dietetic application has recently reported (in a patent) by Degre (2003) who found that polymeric procyanidins (DP > 10) were strong inhibitors of intestinal α-amylase, so they may be useful in dietetics to suppress α-amylase activities, and/or the caloric values of food.

Working with procyanidins from different pine species, we have detected structural variations (Jerez, Pinelo, Sineiro, & Nuñez, 2006) which may be related to different physicochemical and biological properties. The aim of this work is to achieve the fractionation of structurally different procyanidins contained in the aqueous fractions from crude ethanolic extracts of bark of two varieties of pine, *Pinus pinaster* and *Pinus radiata*, and to characterize the different fractions for procyanidin content, degree of polymerization and antiradical activity. The information gained about the properties of the mixtures will help to define their possible applications as food antioxidants and/or as functional components .

2. Materials and methods

2.1. Plant material

Pine (*P. pinaster* and *P. radiata*) barks provided by M. Bouzas Garrido, S.A. (Vedra, A Coruña, Spain) were dried at room temperature for a week. They were then ground in an analytical mill MF 10 IKA-WERKE (Staufen, Germany) to less than 1 mm.

2.2. Reagents

Ethanol, methanol, acetone, toluene, formic acid, hydrochloric acid, sulphuric acid and vanillin were

obtained from Panreac (Moncada i Reixac, Spain). Lipophilic Sephadex LH-20 and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma–Aldrich Química, S.A. (Madrid, Spain). Water, solvents and reagents for analytical RP-HPLC were MilliQ® water, HPLC grade CH_3CN (E. Merck, Darmstadt, Germany) and trifluoroacetic acid (TFA, Fluorochem, Derbyshire, UK), biotech grade distilled in-house. Analytical grade MeOH, cysteamine hydrochloride (Sigma–Aldrich, Steinheim, Germany) and fuming 37% hydrochloric acid (HCl, Merck, Darmstadt, Germany) were used for fraction depolymerisation. RP-HPLC standards: (+)-catechin (Cat), (−)-epicatechin (Ec) and procyanidins (B2) were purchased from Sigma–Aldrich. 4β-(2-Aminoethylthio)- catechin (Cya–Cat), and 4β-(2-aminoethylthio) epicatechin (Cya–Ec) were prepared as described by Torres and Bobet (2001).

2.3. Extraction and fractionation of extracts

Pine bark was extracted in an immersion extractor of 10 × 4.5 cm i.d. The extractor was kept at 37.5 °C by a thermostatted external water bath, ethanol being the solvent. A condenser was fitted to avoid solvent losses. Extraction was accomplished by continuously upward pumping of fresh solvent (17 ml/min) through the cake bed.

The bark extracts obtained were solvent-fractionated, essentially as described by Torres and Bobet (2001). Briefly, the total extract was ethanol-evaporated. The resulting solid was resuspended in water and lyophilized to yield the crude extract, which was defatted with petroleum ether. The residue was dried, suspended in water, acidified with acetic acid and extracted with ethyl acetate. Two fractions were obtained: an organic fraction and an aqueous fraction which contained mainly polymers. The solvent (water saturated with ethyl acetate) was eliminated from the aqueous fraction. The pellet was them resuspended in water and lyophilized to yield fraction FA.

2.4. Separation of the fraction FA on Sephadex LH-20 column

A sample of fraction FA (400 mg/4 ml of 50% methanol) was applied to a 50 × 2.5 cm i.d. Sephadex LH-20 column. Sephadex LH-20 (50 g) was suspended in 50% methanol in water and it was allowed to swell for 24 h before the column was manually packed by elution with the same solvent. The column was eluted with the sequence of solvents mixture shown in Table 1 at a flow rate of 3 ml/min. Samples were collected every 2 min using a fraction collector (Model 2110, BIO-RAD) and their absorbances were measured at 280 nm and 400 nm and only at the latter when the mobile phase contained acetone. Fractions were collected and evaporated under vacuum to remove organic solvents, and dissolved in methanol (4 ml). The column was washed exhaustively with 70% acetone/water (v/v) to obtain fraction F8, containing compounds with a higher DP. The elution of procyanidins was monitored using thin-layer chromatography (TLC).

Table 1
Solvent mixture for the fractionation of FA

P. pinaster vol (ml)	*P. radiata* vol (ml)	Acetone (% vol)	Methanol (% vol)	Water (% vol)	
575	540	0	60	40	(A)
300	240	0	75	25	(B)
360	390	0	90	10	(C)
300	360	10	80	10	(D)
300	300	20	65	15	(E)
300	300	30	40	30	(F)

2.5. TLC

Identification of procyanidins according to their degree of polymerization was effected by TLC under the conditions used by Lea (1978). TLC of fractions was performed on 20 cm × 20 cm silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) eluted with toluene–acetone–formic acid (3:6:1). Separated components were visualized by spraying with 5% vanillin dissolved in ethanol acidified with 10% HCl, followed by heating the plate with a hot air blower. The flavans and procyanidins were revealed as orange to reddish spots.

2.6. Antiradical activity

The antiradical activity of the fractions was evaluated by using the method described by Brand-Williams, Cuvelier, and Berset (1995). A 6.1×10^{-5} M solution of DPPH$^{\cdot}$ in methanol was prepared daily and 980 μl of this solution were mixed with 20 μl of each sample. The initial concentration of DPPH was calculated for every experiment from a calibration curve made by measuring the absorbance at 515 nm of standard samples of DPPH at different concentrations. The equation of the curve was $Abs_{515\,nm} = 11{,}223\ C_{DPPH}$, as determined by linear regression. The results were plotted as the inhibition percentage at 515 nm, defined as $((A_0 - A/A_0) \times 100)$ against the amount of sample divided by the initial amount (μmol) of DPPH. Each point was repeated in triplicate. A dose–response curve was obtained for every extract and fraction. ED_{50} corresponds to microlitres of fraction able to consume half the amount of free radical divided by micromoles of initial DPPH. The antiradical activity (AR) unit was defined as the amount of sample able to consume half the amount of free radical. The results are also expressed as specific antiradical (sAR) activity (units divided by mass of procyanidins).

2.7. Vanillin assay

The analytical method normally employed to estimate the total amount of catechin and procyanidins is the colorimetric measurement after reaction with aromatic aldehydes

such as dimethylamino-cinnamaldehyde (DMACA) or vanillin. Quantification of total flavan-3-ols in each fraction obtained from a Sephadex LH-20 column was performed according to the method described by Sun, Ricardo-da-Silva, and Spranger (1998).

2.5 ml of 25% H_2SO_4 in methanol and 2.5 ml of 1% (w/v) vanillin in methanol were added to 1 ml of each fraction diluted in methanol. The mixture was allowed to stand for 15 min at room temperature, and the absorbance was then measured at 500 nm. The blank was the same as the reaction medium but without vanillin. The total amount of procyanidins in each fraction was expressed as procyanidin B2 equivalents.

2.8. Thiolysis conditions

Fractions from the Sephadex LH-20 column were diluted to 1 mg/ml procyanidin concentration, following the results of vanillin assay.

An aliquot (200 μl) of the fraction was added to the thiolysis mixture (200 μl) which consisted of cysteamine hydrochloride (500 mg) and 37% HCl (200 μl) dissolved in methanol (930 μl). The mixture (400 μl) was kept at 65 °C in a water bath for 15 min.

2.9. Analytical RP-HPLC and estimation of mean procyanidin composition

The size and composition of the procyanidins were estimated from the RP-HPLC analysis of the depolymerised fractions, as described Torres and Selga (2003). Briefly, the terminal flavan-3-ols units were released as such by acid cleavage in the presence of cysteamine whereas the extension moieties were released as the C4 cysteamine derivatives. Thiolysis reaction media (20 μl) were analysed by RP-HPLC on a Smart system (Amersham–Pharmacia Biotech, Uppsala, Sweden) equipped with a μ peak monitor (Amersham–Pharmacia Biotech) and fitted with a 100 × 2.1 mm i.d. μRPC C2/C18 SC 2.1/10 column. Elution: [A] 0.10% (v/v) aqueous TFA, [B] 0.08% (v/v) TFA in water/CH_3CN (1:4), gradient 8–23% [B] over 45 min. The flow rate was 100 μl/min. The detection was done at 214, 280 and 320 nm. The parameters calculated were: mean degree of polymerization (mDP) = total nmol/nmol terminal units, mean molecular weight (mMW) = total mass/nmol terminal units.

3. Results and discussion

The starting-point of this work was the processing of crude extracts, obtained in previous works from the ethanolic extraction of *P. pinaster* and *P. radiata* barks. The mean degrees of polymerisation for these extracts of *P. pinaster* and *P. radiata* were 7.0 and 5.4, respectively, the specific antiradical powers being 34.3 and 31 AR(antiradical) units/mg polyphenols (Jerez et al., 2006). Crude extracts were partially separated, essentially as previously described (Torres & Bobet, 2001), rendering a fraction, FOW, with compounds soluble in both ethyl acetate and water, and another fraction, FA, containing species soluble in water but not in organic solvents. This fraction, named aqueous fraction from now on, is analysed in this paper.

Fig. 1 shows the HPLC chromatogram for the aqueous fraction of *P. pinaster*, pFA; it basically consists of a broad hump, which indicates the presence of a mixture of procyanidins with different DPs (profile for *P. radiata* is similar). When pFA was depolymerised by thiolysis with toluene-α-thiol (Jerez, 2003), the resolution of this peak showed the existence of (+)-catechin, (−)-epicatechin and the corresponding benzyl-thioethers. As the entire FAs are complex, more homogeneous procyanidin fractions (with different degrees of polymerisation suitable for different applications) can be obtained by fractionating FAs on a Sephadex LH-20 column. Resulting fractions will be later

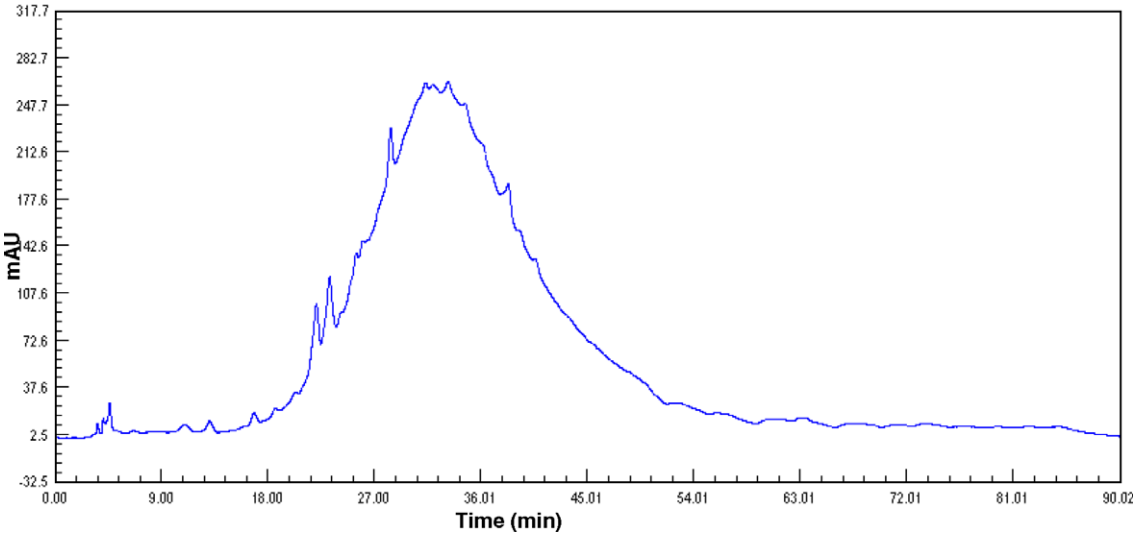

Fig. 1. Chromatogram of the aqueous fraction FA from *P. pinaster*.

depolymerised for characterization. Table 1 summarises the elution conditions used. This mixture was previously used by other authors (Taylor et al., 2003) for fractioning hop procyanidins. Under these conditions, low molecular weight (MW) compounds are eluted first and bulkier procyanidins are recovered last.

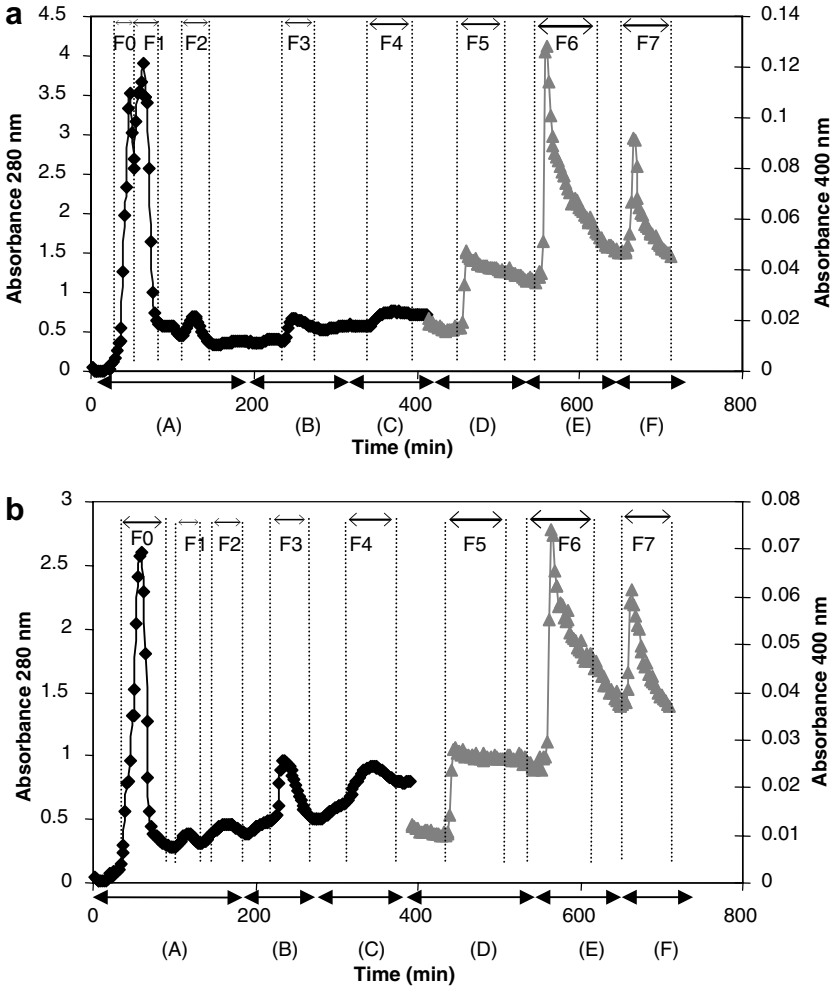

Fig. 2. Chromatographic profile obtained by fractionation of fraction FA on Sephadex LH-20 column: (a) *P. pinaster* and (b) *P. radiata*.

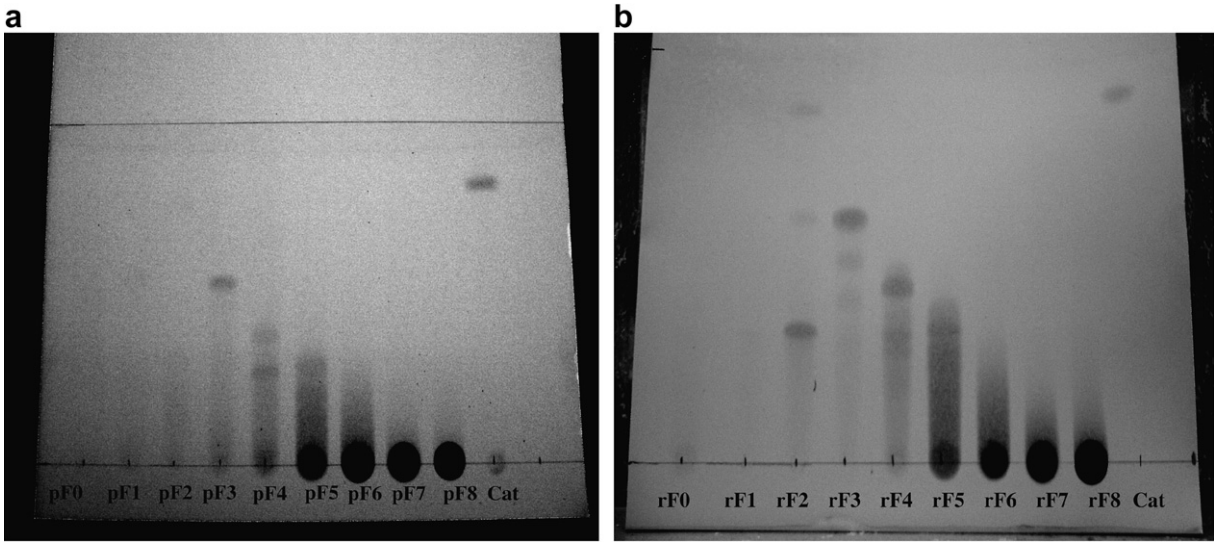

Fig. 3. TLC of fractions from Sephadex LH-20 column runs in one dimension with (toluene/acetone/formic acid) as solvent: (a) *P. pinaster* and (b) *P. radiata*.

Fig. 2a and b shows the elution profiles which were obtained by measuring the absorbance of the fractions collected at intervals of 2 min in an UV-V Jasco V-530 spectrophotometer. Fractions under each peak were pooled into 8 fractions, F0–F7. Procyanidins of higher MW remained tightly adsorbed at the top of the column. Because the chromatographic mode is adsorption rather than exclusion, the separation can be incomplete (Yanagida et al., 2000). Washing with 70% acetone resulted in the recovery of high mDP procyanidins. This washing was carried out, instigating the recovery of fraction F8. Acetone was chosen because the carbonyl oxygen serves as a strong H-bond acceptor and allows displacement of bound polymeric phenols from Sephadex LH-20.

TLC of each fraction was performed to obtain preliminary information concerning their procyanidin content (DP), following the vanillin assay (Fig. 3a and b). In the first fractions, pF0, pF2 for *P. pinaster* and rF0, rF1 for *P. radiata*, no spots were observed, indicating the absence of flavan-3-ols, either as monomers or as their derivatives, and these fractions were not included in the subsequent analyses. In any case these fractions may contain phenolic acids and taxifolin (dihydroquercetin, present basically in fractions soluble in both ethyl acetate and water), frequent in pine bark (Wood et al., 2002). (+)-catechin was only observed, as traces, in rF2. The developed spots in fractions pF3–pF4 and rF2–rF4 corresponded to procyanidins of low molecular weight. For the other fractions, the spots remained on the base-line, indicating that they included bulky hydrophilic high molecular weight compounds.

Each one of the obtained fractions containing procyanidins was thiolysed with cysteamine, which was preferred to toluene-α-thiol for being more user-friendly and much less toxic (Torres & Selga, 2003). Figs. 4a and b and 5a and b

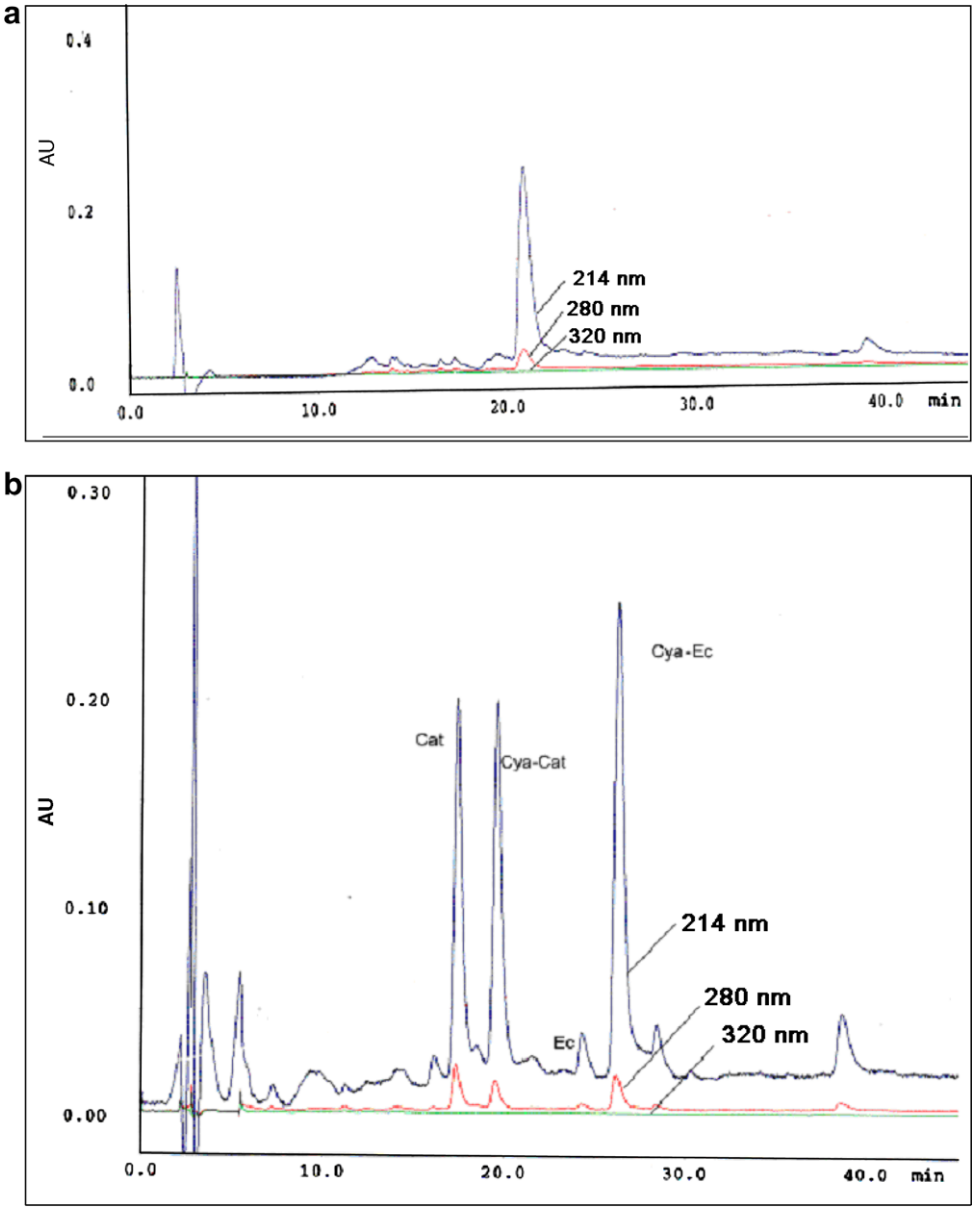

Fig. 4. RP-HPLC chromatograms of fraction F3 from *P. pinaster* (pF3), obtained by fractionating FA: (a) raw fraction and (b) thiolysed fraction.

show the chromatogram of F3 of the barks from the two pine varieties. In agreement with our previous results, *P. radiata* contained more procyanidins than *P. pinaster*. The profile after thiolysis indicated that (+)-catechin was the main terminal unit in both cases. Regarding the extension units, the situation is different. *P. radiata* contained almost exclusively catechin units whereas *P. Pinaster* included both epicatechin and catechin extension units (Fig. 4b).

In Tables 2 and 3, the structural compositions of the different fractions are indicated. For all *P. pinaster* fractions and whole pFA, epicatechin was the main extension unit, with a ratio of 3–6 with respect to catechin, except for pF3, for which the ratio was 1.2.

P. radiata fractions were also consistent with whole rFA, with a proportion catechin/epicatechin extension units of 1.2. Again, rF3 was the exception to the rule, the ratio being 10.8. In this case, the mean composition of rF2 was also different, because epicatechin predominated as the extension unit. The presence of catechin as an abundant extension unit in *P. radiata* may influence the physicochemical properties of procyanidins, which will be more hydrophobic than those from *P. pinaster*. This fact can be of interest for the possible antioxidant activity in oils, emulsions and biological systems.

Tables 4 and 5 summarise the mean structural parameters and activity of the entire FAs and eluted fractions (procyanidin content, antiradical power, mDP and

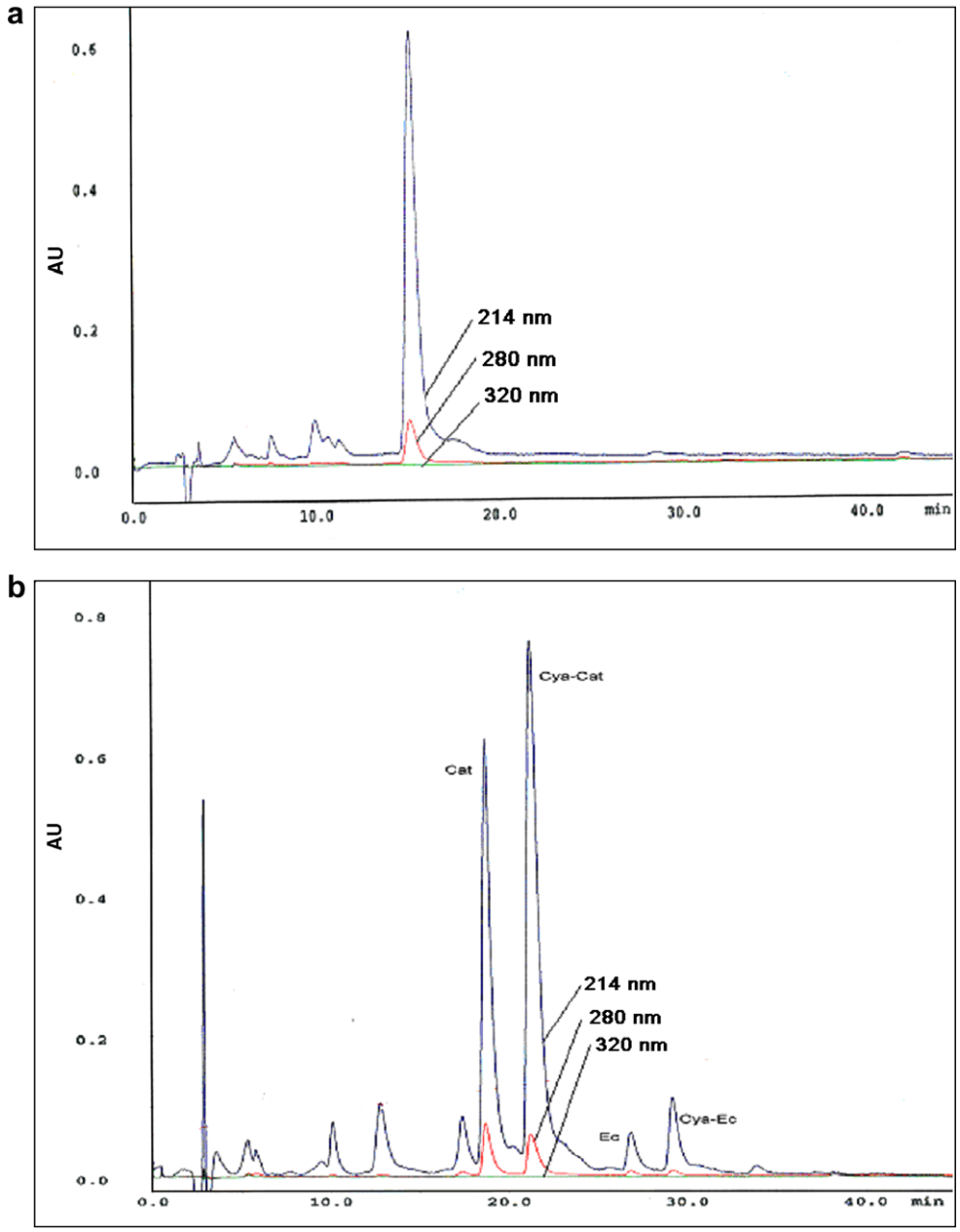

Fig. 5. RP-HPLC chromatogram of fraction F3 from *P. radiata* (rF3), obtained by fractionating FA: (a) raw fraction and (b) thiolysed fraction.

Table 2
Structural composition of fractions from FA of *P. pinaster*, determined by HPLC following thiolysis degradation

Fraction	Proportion of terminal units (%)		Proportion of extension units (%)	
	Catechin	Epicatechin	Aminoethylthio catechin	Aminoethylthio epicatechin
pFA	8.1 ± 0.1	1.4 ± 0.2	13.5 ± 0.4	77.0 ± 0
pF3	35.9 ± 0.3	3.8 ± 0	26.9 ± 1.0	33.5 ± 0.7
pF4	20.5 ± 0.6	1.6 ± 0.3	18.0 ± 0.3	60.0 ± 0
pF5	13.1 ± 0.3	0.8 ± 0	14.5 ± 0.7	71.7 ± 0.9
pF6	7.8 ± 0.4	nd	12.4 ± 0.2	79.6 ± 0.4
pF7	5.5 ± 0.1	nd	12.6 ± 0.1	81.9 ± 0.1
pF8	4.6 ± 0.2	nd	12.9 ± 0.5	82.7 ± 0.2

Table 3
Structural composition of fractions from FA of *P. radiata*, determined by HPLC following thiolysis degradation

Fraction	Proportion of terminal units (%)		Proportion of extension units (%)	
	Catechin	Epicatechin	Aminoethylthio catechin	Aminoethylthio epicatechin
FA	10.4 ± 0.6	2.3 ± 0.2	44.2 ± 0.4	43.4 ± 0.17
F2	40.8 ± 3.2	7.9 ± 0.9	21.7 ± 1.2	29.8 ± 1.1
F3	44.7 ± 1.5	3.4 ± 0.7	47.5 ± 0.4	4.4 ± 0.4
F4	25.8 ± 0.9	2.4 ± 0.3	48.3 ± 1.0	23.6 ± 0.2
F5	15.2 ± 1.2	0.4 ±0.5	45.8 ± 0.3	38.7 ± 0.4
F6	9.9 ± 0.1	1.1 ± 0.1	49.1 ± 0.1	39.9 ± 0.2
F7	8.0 ± 0.2	nd	50.4 ± 0.4	41.5 ± 0.4
F8	6.3 ± 0.3	0.6 ± 0.1	53 ± 0	40 ± 0

mMW), which were calculated from the thiolysis data. Procyanidins for FAs represent 65% of total polyphenols in pinaster, increasing to 87% for radiata. Up to fraction 4, eluted with water–methanol, mDP were similar for the two varieties; *P. radiata* was richer in procyanidins and was more active. The antiradical activity per mass (specific antiradical activity, sAR) is more suitable for interpreting the effect of polymerisation on the scavenging activity. According to this index, values are similar for pF3, pF4, and not very different from that of the total pFA. In contrast, sAR for the whole rFA was much higher than sAR for rF2–rF4. From F5 on, fractions eluted with acetone, the situation is different; pF5 and rF5, with mDP about 7, showed the highest sAR, and the subsequent fractions from *P.radiata* presented a gradual decrease in this parameter, down to values similar to those of the first fractions. In *P. pinaster*, the behaviour did not follow exactly the same trend; pF6–pF8 showed similar sAR values, around 56. This different pattern may be related to the differences detected in the extension units for both varieties (Tables 2 and 3). Fractions with mDP above 7 from *P. pinaster* contained mostly epicatechin as extension unit while *P. radiata* oligomers contained both catechin and epicatechin in similar proportions. This fact may influence the antiradical effectiveness, because the stereoposition of the phenolic OH group in C3 is involved in the oxidation mechanism, especially by radicals.

The increase of antiradical power of flavanols with mDP up to 7 can be explained by the electron delocalisation through intramolecular links among monomers, because C4–C8 confers a very tight conformation but, at a given point, with new units the steric hindrance can be decisive (Gaulejac et al., 1999). Gaulejac et al. (1999) found an increase in activity for procyanidins from 1 to 4 units. In the present work, we have recorded an increase up to 6–7 units and from there, either a stabilisation (*P. pinaster* 12–22 mDP) or a drop in sAR (*P. radiata* 9–15 mDP).

When procyanidins incorporate gallate, tendencies are diverse. The trend is an increase of antiradical power until DP = 3; if we compare values for F3 (Tables 3, 4) with an analogous fraction of grape pomace with 25% galloylation (Pazos, 2005), antiradical power for grape is 1.5 times

Table 4
Characteristics of fractions from FA of *P. pinaster*

Fractions	mDP	mMW	Procyanidins (mg)	Total AR activity units	AR activity units/mg procyanidins (sAR)
pFA (400 mg)	10.6 ± 0.4	3067 ± 119	139 ± 3.0	5488	39.5
pF3	2.5 ± 0	731 ± 6	1.1 ± 0.1	41.9	38.2
pF4	4.6 ± 0.1	1313 ± 18	2.9 ± 0.3	106.7	36.3
pF5	7.2 ± 0.1	2090 ± 44	8.8 ± 0.2	561.8	63.5
pF6	12.8 ± 0.4	3713 ± 110	24 ± 0.4	1360.5	56.7
pF7	18.2 ± 0.3	5258 ± 91	10.5 ± 0.2	580.6	55.2
pF8	22.2 ± 1.1	6425 ± 303	22.4 ± 0.5	1261.8	56.3

Table 5
Characteristics of fractions from FA of *P. radiata*

Fractions	mDP	mMW	Procyanidins (mg)	Total AR activity units	AR activity units/mg procyanidins (sAR)
rFA (400 mg)	7.9 ± 0.2	2278 ± 56	166 ± 4	9650	58.1
rF2	2.1 ± 0.1	598 ± 28	2.0 ± 0.3	84.6	41.2
rF3	2.1 ± 0.1	603 ± 9	2.2 ± 0.1	99.5	45.8
rF4	3.6 ± 0.2	1033 ± 45	3.8 ± 0.2	187.8	49.9
rF5	6.5 ± 0.3	1875 ±88	13.0 ± 0.4	851.1	65.4
rF6	9.2 ± 0.1	2647 ± 13	26.9 ± 0.1	1428.6	53.1
rF7	12.6 ± 0.4	3651 ± 110	11.9 ± 0.3	571.4	48.0
rF8	14.6 ± 0.5	4226 ± 137	50.6 ± 0.6	2353	46.5

higher than that for rF3, and 3 times higher than pF3. In the literature, we have not found references to procyanidins with high DP and galloylated, although Gaulejac et al. (1999) pointed out that, from trimers on, gallate may induce the adoption of a very compact "box structure", which lowers ability to scavenge free radicals. In agreement with the literature, we did not detect the presence of any gallate ester in pine bark extracts. None of the procyanidins absorbed at 320 nm and the thiolysis did not yield any galloylated monomer or conjugate (e.g. Figs. 4 and 5). In any case, since galloylation does not appear to be relevant for protection against oxidation in emulsions (Touriño et al., 2005), pine bark may become an excellent source of antioxidants, rich in procyanidins and easy to handle. Moreover, because aqueous fractions are often discarded, this paper may also be a new contribution towards the integral use of plants.

Acknowledgements

We thank Spanish Ministry MCyT for Project PPQ2003-06602-C04-01 y 02.

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