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A comparison between bark extracts from *Pinus pinaster* and *Pinus radiata*: Antioxidant activity and procyanidin composition

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Abstract

The composition and antiradical activity of procyanidins from the bark of two kinds of pine, *Pinus pinaster* and *Pinus radiata*, were compared. Both the total bark extract and the fraction soluble in both water and ethyl acetate (**OW**) were evaluated, because of their promising results in previous experiments.

Results showed that *P. radiata* bark was richer in total phenols and also in procyanidins, catechin always being the main unit, so terminal as extensional. For *P. pinaster,* epicatechin was the predominant extension unit. The mean degree of polymerization (mDP) was higher for the latter. Interestingly, opposite results were encountered for the corresponding **OW** fractions, where *P. radiata* showed a mDP of 2.9 vs. 2.3 of *P. pinaster*. It was also found that the higher the mDP the higher was the specific antiradical activity. The different procyanidin composition and specific antiradical activity of the two kinds of barks, and particularly their **OW** fractions, may lead to the design of efficient natural antioxidants with application in the food industry.

Keywords: *Pinus pinaster*; *Pinus radiata*; Procyanidins; Antioxidant activity; Fractionation; Degree of polymerization

1. Introduction

Pine bark was, until a few years ago, an inconvenient residue for the wood industry, with a limited use. Nowadays, it is employed as vegetal substrate and combustible. Interestingly pine bark is a rich source of natural polyphenols, compounds which have attracted increasing attention in the fields of nutrition, health and medicine. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes and tannins, are important in the plants for normal growth development and defence against infection and injury (Kähkönen et al., 1999). The procyanidins, one subclass of proanthocyanidins, are mixtures of oligomers and polymers, consisting of (+)-catechin and/or (−)-epicatechin units linked mainly through C4 → C8 and/or C4 → C6 bonds. These flavan-3-ols units can be doubly linked by a C4 → C8 bond and an additional ether bond between O7 → C2 (Gu et al., 2002). They are the most widespread polyphenols in plants after lignins, and can be found in leaves, fruits, barks or roots, often in high concentrations.

Recently, procyanidins have received considerable attention owing to their pharmacological effects, in particular on atherosclerosis, and their radical-scavenging ability (Escribano-Bailón, Gutiérrez-Fernández, Rivas-Gonzalo, & Santos-Buelga, 1992). Pycnogenol is an extract of the bark of *P. pinaster* composed of a mixture of flavonoids, mainly procyanidins, phenolic acids and taxifolin (Wood, Senthilmohan, & Peskin, 2002) and it is probably the most studied phenolic tree extract. Pycnogenol is now utilized throughout the world as a nutritional supplement and as a phytochemical remedy for various diseases, having cardiovascular benefits, such as a vein-relaxant activity and the ability to enhance the microcirculation by increasing

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capillary permeability (Packer, Rimbach, & Virgili, 1999; Rohdewald, 2002).

Polyphenolic extracts and fractions have also a potential use in the food industries as alternative options to synthetic BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole) and TBHQ (*tert*-butyl hydroquinone). For instance, it has been shown that procyanidin fractions from grape pomace protect corn and fish oil in water emulsions as well as frozen fish muscle from oxidation of the functional polyunsaturated fatty acids. The efficacy of this protection varies with significant parameters, such as degree of polymerisation and percentage of galloylation (Torres et al., 2002). We have recently shown that Pine (*P. pinaster*) bark is an alternative source of procyanidin fractions with possible application as food antioxidants (Touriño et al., 2005). Compared to grape pomace, pine procyanidins are devoid of gallate esters; since the latter are involved in the regulation of certain cellular functions (cell cycle, apoptosis) (Liang, Lin-shiau, Chen, & Lin, 1997; Wang, Song, Guo, & Tian, 2003), pine procyanidins might be more suitable for food applications from the safety standpoint. More in favour of pine bark as an alternative source of procyanidins is the observation that gallates do not appear to make much difference in lipid protection in emulsion (Touriño et al., 2005).

The size of procyanidins, expressed as degree of polymerization (DP), is one of the most important property; it appears that the degree of polymerization increases the antioxidant capacity (Hagerman et al., 1998; Saint-Cricq de Gaulejac, Provost, & Vivas, 1999). Classically, total procyanidin content is determined by colorimetric methods, butanol/HCl assay and the vanillin assay but, at present, methods based on acid-catalyzed degradation in the presence of nucleophiles (phloroglucinol, toluene-α-thiol or cysteamine hydrochloride) allow the estimation of the constitutive units and degree of polymerization of procyanidin extracts. The degradation products can be analyzed by reversed-phase HPLC, and the results will provide information on the nature of extension and terminal units and on the degree of polymerization (Guyot, Marnet, & Drilleau, 2001; Torres & Selga, 2003). Acid depolymerization in the presence of a nucleophile is useful for the analysis of plant samples such as pine bark rich in proantocyanidins (PA) showing C4–C6 and C4–C8 interflavanic bonds. Apart from the quantitative analysis of total PA, acid depolymerization gives information about their structure. In contrast with colorimetric methods, interferences with other plant constituents are avoided, due to nonambiguous identification of the PA-derived products. In comparison to butanol/HCl depolymerization, the reaction largely preserves the stereochemistry at the C2–C3 positions of the polymer units. Finally, the use of a nucleophile limits the occurrence of side reactions that could affect the recovery yields of the products.

We are interested in identifying byproducts from the agricultural and forestry industries, and finding the right application for every material. To explore the suitability of different species of *Pinus* as sources of procyanidins we have compared the barks from *P. pinaster* and *P. radiata*, which may be collected and treated separately in sustainable forest exploitation. The specific goals of this work were to estimate the procyanidin content and composition of the bark from two varieties (*P. pinaster* and *P. radiata*) of pine and of their fractions soluble in both ethyl acetate and water (**OW** fractions) and to compare these results with the antiradical activity of the mixtures.

2. Materials and methods

2.1. Materials

The raw plant materials provided by Manuel Bouzas, S.A. (Vedra, A Coruña, Spain) were dried at room temperature for a week. They were then ground in a analytical mill MF 10 IKA-WERKE (Staufen, Germany) to less than 1 mm. Ethanol, methanol and Folin–Ciocalteu reagent were obtained from Panreac (Montcada i Reixac, Spain), and 2,2-diphenyl-1-picrylhydrazyl was purchased from Sigma–Aldrich Química, S.A. (Madrid, Spain).

Water, solvents and reagents for analytical RP-HPLC were Milli-Q® 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 hydrochloric acid 37% (HCl, Merck, Darmstadt, Germany) were used for bark and fraction depolymerisation. RP-HPLC standards, (+)-catechin (Cat) and (−)-epicatechin (Ec), were purchased from Sigma-Aldrich; 4β-(2-aminoethylthio)-catechin (Cya-Cat), and 4β-(2-aminoethylthio) epicatechin (Cya-Ec) were prepared as described (Torres & Bobet, 2001).

2.2. Phenolics content and antiradical activity: continuous extraction

Pine bark (13 g) were extracted in an immersion extractor of 4.5 cm i.d. and 10 cm height. 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.

2.3. Fractionation of extracts

The obtained bark extracts, **pBark** (ethanolic extract from *P. pinaster*) and **rBark** (ethanolic extract from *P. radiata*) were fractionated as described by Torres and Bobet (2001), for obtaining fractions **pOW** (*P. pinaster* fraction) and **rOW** (*P. radiata* fraction), soluble in both water and ethyl acetate. To render fOW, the total extract, after adding ethanol to bark, was solvent-evaporated, resuspended in water, acidified with acetic acid, and extracted with ethyl

acetate. Two fractions were obtained; one of them, the aqueous fraction, (FA), was discarded for this work. The organic fraction was solvent-evaporated, resuspended in water and filtered. The lyophilized filtrate was the fraction OW. It contained procyanidins of low polymerization degree (basically oligomers).

2.4. Determination of total phenolic content

The total polyphenol content was determined by the Folin–Ciocalteu method, as modified by Singleton and Rossi (1965). The Folin–Ciocalteu reagent was diluted 1:10 before use. All the polyphenolic samples were dissolved in distilled water before being assayed. Diluted Folin–Ciocalteu reagent (2.5 ml) was added to 0.5 ml of aqueous phenolic-containing samples. After an interval of 3 min, 2 ml of 7.5% sodium carbonate was added. The absorbance was measured at 765 nm after 1 h of incubation at room temperature. A mixture of water and reagents was used as a blank. The content of phenolics was expressed as gallic acid equivalents.

2.5. Antiradical activity

The antiradical activities of the extracts and fractions were 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 was 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 extract or micrograms of fraction able to consume half the amount of free radical divided by micromoles of initial DPPH. The antiradical activity unit was defined as the amount, either weight or volume, of sample able to consume half the amount of free radical. The results are also expressed as specific antiradical activity (units divided by milligrammes of polyphenols).

2.6. Sample preparation for analytical RP-HPLC and estimation of mean procyanidin composition

2.6.1. Fractions

The fraction lyophilisates (10 mg) were dissolved in MeOH (1 ml). An aliquot (200 μl) was added to the thiolysis mixture (200 μl), which consisted of cysteamine hydrochloride (50 mg) and 37% hydrochloric acid (20 μl) dissolved in MeOH (930 μl). The mixture (400 μl) was kept at 65 °C for 15 min. Then the reaction was quenched with 0.1% (v/v) aqueous TFA (1.6 ml) and the mixture analyzed by RP-HPLC. The depolymerization reaction is illustrated in Fig. 1. Extension units are released as the 2-aminoethylthio-derivatives while the terminal units are released as underivatized catechin monomers. The same method was applied directly to samples already in solution.

2.7. Analytical RP-HPLC and procyanidin composition

The size and composition of the procyanidins were estimated from the RP-HPLC analysis of the depolymerized mixtures by thiolysis with cysteamine as described (Torres

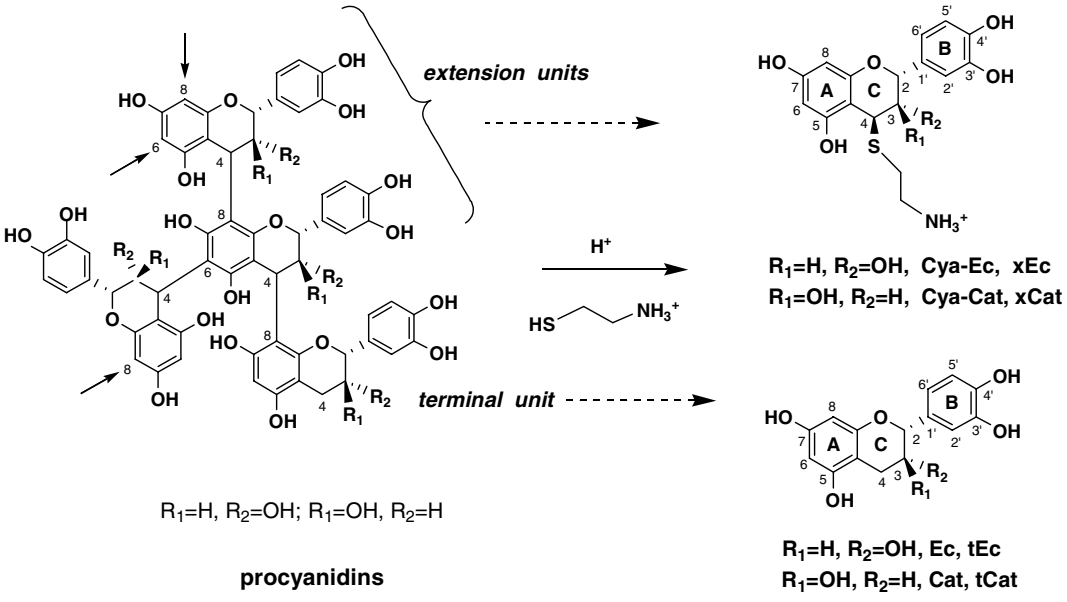

Fig. 1. Depolymerization of procyanidins in the presence of cysteamine. Structure of the starting polymers and the resulting cleaved terminal and extension units.

& 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 C-4 cysteamine derivatives. The cleaved mixtures were analyzed 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 200 μl/min. The detection was done at 214 nm. The parameters calculated were: mean degree of polymerization (mDP) = total nmoles/nmoles terminal units, and conversion (CON). For whole pine bark, bCON was the ratio (mg/g) between the total amount of depolymerized moieties (or amount of procyanidins) estimated by RP-HPLC and the amount of pine bark. For **OW** fractions, fCON was the ratio (μg/mg) between the total amount of procyanidins estimated by RP-HPLC and the amount of fraction injected.

3. Results and discussion

In the present work, we have compared the phenol content, antiradical activity and procyanidin content, as well as the composition of both bark extracts from Galician *P. pinaster* and *P. radiata* and their fractions soluble in both ethyl acetate and water. Polyphenolic fractions, such as those described here, usually contain monomeric polyphenols and oligomeric procyanidins (Packer et al., 1999), while the whole bark includes procyanidins of higher molecular weight.

Table 1 shows that the bark from radiata, after 12 min of extraction, contained more polyphenols than the bark from pinaster (12% vs. 7% referred to initial extracted weight). Once analyzed, the bark extracts were fractionated to obtain the **OW** fraction containing flavan-3-ols and oligomeric procyanidins. It can be seen that the yields were also higher for **rOW** (204 vs. 90 mg), the polyphenolic content being 3 times higher. In the two last rows of Table 1, the total AR units (both overall as specific) are shown. The extracts with more antiradical activity were those from *P. radiata*, both bark and **OW** fraction. However, specific AR values appears to be similar in all cases. The highest value corresponds to **pBark**, followed by **rOW**.

Our results (b**CON**) indicate that the bark from *P. radiata* contained higher amounts of procyanidins than the bark of *P. pinaster* (Table 2). The mean size of these polymers, expressed as mDP, was higher for *P. pinaster*. The mDP of the **OW** fractions followed an opposite trend. *P. radiata* **rOW** gave higher mDP than *P. pinaster* **pOW** (Table 3). During the preparation of **OW** fractions, the bulkier more water-soluble polymers were left behind and the ethyl acetate-soluble medium sized (mDP around 3) oligomers were obtained. The results indicate that *P. radiata* bark contained more hydrophobic oligomers (**rOW**, soluble in ethyl acetate) bulkier (mDP 2.9) than those from *P. pinaster* (**pOW**, mDP 2.2). In any case, a higher mDP appears to be related to higher specific AR values independently of the procyanidin source. Accordingly, **pBark**, with a mDP higher than **rBark**, showed higher specific AR, and **rOW**, with higher mDP than **pOW**, showed higher specific AR.

The distribution of terminal and extension subunits provided additional information (Table 4) on the differences between procyanidins from the two different origins. While the main terminal unit was always catechin (Cat), epicatechin was the predominant extension unit in *P. pinaster* whereas catechin was predominant in *P. radiata*, from

Table 2
Procyanidin composition and content of whole bark

Sample	mDP	bCON
pBark	7.6 ± 0.3	38 ± 6
rBark	5.3 ± 0.1	60 ± 10

Results are expressed as the mean value ± SEM (standard error of the mean).

Table 3
Procyanidin composition and content of **OW** fractions

Sample	mDP	fCON
pOW	2.3 ± 0.1	17 ± 2
rOW	2.9 ± 0.1	36 ± 6

Results are expressed as the mean value ± SEM (standard error of the mean).

Table 4
Distribution of procyanidin terminal and extension units of whole bark and fractions

Sample	% tCat	% xCat	% tEc	% xEc
pBark	13 ± 1	16 ± 3	n.o.	71 ± 3
pOW	40 ± 2	13 ± 2	5 ± 1	42 ± 2
rBark	19 ± 1	59 ± 1	n.o.	22 ± 1
rOW	34 ± 1	49 ± 1	5 ± 1	12 ± 1

Results are expressed as the mean value ± SEM (standard error of the mean).
tCat, terminal catechin; xCat, extension catechin (Cya-Cat, Fig. 1); tEc, terminal epicatechin; xEc, extension epicatechin (Cya-Ec, Fig. 1); n.o., not observed.

Table 1
Total phenolics and antiradical activities of the extracts and fractions

Sample	Total (wt/vol)	Total phenols (mg)	Total AR activity units ($\times 10^{-4}$)	AR activity units/mg polyphenols
pBark	200 ml	890	3.05	34.31
rBark	200 ml	1610	5.00	31
pOW	90 mg	42.3	0.12	29.39
rOW	203.8 mg	130.4	0.43	33.15

either whole bark or **OW** fractions. The presence of catechin moieties as extension units must have had some influence on the physicochemical properties of the procyanidins, resulting in differences in composition of **OW** fractions upon solvent extraction. It might be suggested that catechin-rich procyanidin oligomers from *P. radiata* are more hydrophobic than those from *P. pinaster* and are more efficiently extracted with ethyl acetate. Alternatively, our results might just be denoting that **rBark** contains oligomeric procyanidins with mDP around 3 that are absent in **pBark**. Whatever the explanation is, the fact that **rOW** contains bulkier hydrophobic oligomers may have important implications for the possible antioxidant activity of the fractions in oils, emulsions and other food and biological systems. In fact, the capacity of different antioxidants to be located at the oil–water interfaces appears to play a role in oil protection even which is greater than their raw free radical-scavenging power (Frankel, 2001; Frankel & Meyer, 2000). We have postulated that small (2–4 catechin units) procyanidin oligomers may be flexible enough to show an amphipathic (hydrophobic–hydrophilic) behaviour at the interfaces (Torres et al., 2002). This does not appear to be the case for bulkier polymers, which are highly water-soluble and insoluble in organic solvents and oils. The results presented here, showing that *P. radiata* contains more hydrophobic oligomers than *P. pinaster* may open the way to the preparation of fractions active in emulsion-like environments in good yields. According to Potter's polar paradox (Porter, 1993; Porter, Black, & Drolet, 1989), apolar (hydrophobic) antioxidants are particularly effective in water environments (e.g. oil-in-water emulsions), probably because they tend to be located at the interface. Thus, radiata would be more suitable than pinaster as a source of amphipathic antioxidants. We are currently addressing this point in our laboratories. Considering that certain fractions from pine bark present the additional advantage of being highly efficient with no content of gallate moieties, which have been proved to influence the cell cycle, *P. radiata* bark appears to be a promising source of procyanidins with application as antioxidants in the food industry.

In conclusion, *P. radiata* is a richer source of procyanidins than is *P. pinaster*. The procyanidin composition of the fraction (**OW**, mainly catechin monomers and procyanidin oligomers), soluble in ethyl acetate and water, presents significant differences: the mean degree of polymerisation is higher for radiata (2.9 vs. 2.3) and this source is richer in catechin moieties. Moreover, procyanidins from radiata appear to be more hydrophobic than those from pinaster. This may lead to the design of antioxidant products active in emulsion.

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References

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wiss und Technology, 28*, 25–30.

Escribano-Bailón, T., Gutiérrez-Fernández, Y., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (1992). Characterization of procyanidins of *Vitis vinifera* variety tinta del pais grape seeds. *Journal of Agricultural and Food Chemistry, 40*, 1794–1799.

Frankel, E. N. (2001). Interfacial lipid oxidation and antioxidation. *Journal of the Oleo Science, 50*, 387–391.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Gu, L., Kelm, M., Hammerstone, J. F., Beecher, G., Cunningham, D., Vannozzi, S., et al. (2002). Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *Journal of Agricultural and Food Chemistry, 50*, 4852–4860.

Guyot, S., Marnet, N., & Drilleau, J.-F. (2001). Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. *Journal of Agricultural and Food Chemistry, 49*, 14–20.

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., et al. (1998). High molecular weight plant poliphenolics (Tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry, 46*, 1887–1892.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J-P., Pihlaja, K., Kujala, T. S., et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954–3962.

Liang, Y. C., Lin-shiau, S. Y., Chen, C. F., & Lin, J. K. (1997). Suppression of extracellular signals and cell proliferation through EGF receptor binding by (−)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *Journal of Cellular Biochemistry, 67*, 55–65.

Packer, L., Rimbach, G., & Virgili, F. (1999). Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. *Free Radical Biology and Medicine, 27*, 704–724.

Porter, W. L. (1993). Paradoxical behavior of antioxidants in food and biological systems. In G. M. Williams (Ed.), *Antioxidants: chemical, physiological, nutritional and toxicological aspects* (pp. 93–122). Princeton, NJ: Princeton Scientific.

Porter, W. L., Black, E. D., & Drolet, A. M. (1989). Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *Journal of Agricultural and Food Chemistry, 37*, 615–624.

Rohdewald, P. (2002). A review of the French maritime pine bark extract (Pycnogenol (R)), a herbal medication with a diverse clinical pharmacology. *International Journal of Clinical Pharmacology and Therapeutics, 40*, 158–168.

Saint-Cricq de Gaulejac, N., Provost, C., & Vivas, N. (1999). Comparative study of polyphenol scavenging activities assessed by different methods. *Journal of Agricultural and Food Chemistry, 47*, 425–431.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Torres, J. L., & Bobet, R. (2001). New flavanol-derivatives from grape (*Vitis vinifera*) byproducts. Antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. *Journal of Agricultural and Food Chemistry, 49*, 4627–4634.

Torres, J. L., & Selga, A. (2003). Procyanidin size and composition by thiolysis with cysteamine hydrochloride and chromatography. *Chromatography, 57*, 441–445.

Torres, J. L., Varela, B., García, M. T., Carilla, J., Matito, C., Centelles, J. J., et al. (2002). Valorization of grape (*Vitis vinifera*) byproducts. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. *Journal of Agricultural and Food Chemistry, 50*, 7548–7555.

Touriño, S., Selga, A., Jiménez, A., Juliá, L., Lozano, C., Lizárraga, D., et al. (2005). Procyanidin fractions from pine (*Pinus pinaster*) bark: radical-scavenging power in solution, antioxidant activity in emulsion and antiproliferative effect in melanoma cells. *Journal of Agricultural and Food Chemistry, 53*, 4728–4735.

Wang, X., Song, K.-S., Guo, Q.-X., & Tian, W.-X. (2003). The galloyl moiety of green tea catechins is the critical structural feature to inhibit fatty-acid synthase. *Biochemical Pharmacology, 66*, 2039–2047.

Wood, J. E., Senthilmohan, S. T., & Peskin, A. V. (2002). Antioxidant activity of procyanidin-containing plant extracts at different pHs. *Food Chemistry, 77*, 155–161.