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Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*)

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Abstract

Lignocellulosic residues, such as pine sawdust and almond hulls, were solvent-extracted under different experimental conditions to optimize the yield of polyphenolic antioxidant compounds, which were quantified. The antioxidant power of extracts was evaluated by ability to scavenge the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical. Both materials were found to be important sources of phenolic antioxidants, although the efficiency of the extraction varied with the experimental conditions. Among the three solvents used (ethanol, methanol and water), ethanol was the most favourable for total extractables, although methanol was more selective for extracting polyphenolics. For these latter, pine sawdust offered the best results, with a 3–10 times higher (0.1122 g/100 g in dry basis) total phenolics content than almond hulls but, despite this, phenols from hulls showed a higher antioxidant capacity (58 vs 34% of inhibition).

Keywords: Antioxidant capacity; Phenols; Almond hull; Pine sawdust; Extraction

1. Introduction

Over the past several years, several scientific papers have focused the polyphenolic content of different lignocellulosic residues. High amounts of phenolics, mainly tannins, such as rhamnetin, quercetin and kaempherol aglycones, have been reported in almond hulls, representing 4.5% of total hull weight (Cruess, Kilbuck, & Hahl, 1947; Shahidi, 2002). Other phenolic compounds, such as chlorogenic and benzoic acid derivatives were also found in lower quantities (Shahidi, 2002; Takeoka & Dao, 2000). In pine residues, procyanidin oligomers are the predominant phenolics (Pietta, Simonetti, & Mauri, 1998; Wood, Senthilmohan, & Peskin, 2002).

Great interest has been recently focused on the addition of polyphenols to foods and biological systems, due to their well-known abilities to scavenge free radicals, i.e. antioxidant power. The generation of free radicals plays an important role in the progression of a great number of pathological disturbances, such as atherosclerosis (Steinberg, 1992), brain dysfunction (Gordon, 1996) and cancer (Ames, 1983; Feskanish et al., 2000; Michels et al., 2000) and also has diverse effects on inflammatory diseases (Decharneux, Dubois, Beauloye, Warriaux-de Coninck, & Wattiaux, 1992).

Effects of bioflavonoids, extracted from the pine, on free radical formation have already been investigated in murine macrophage cell lines, such as strong scavenging activities against reactive oxygen species (Cho et al., 2000), so enhancing the antioxidant defences. Other studies demonstrate that reactive nitrogen species, generated with different kinetics and mechanisms, impair gluthathione levels in endothelial cells (Rimbach, Virgili, Park, & Packer, 1999). On the other hand, the beneficial effects of almond phenolics on the protection of DNA and inhibition of human LDL (low-density lipoproteins) oxidation have been also reported (Shahidi, 2002).

Due to these facts, it would be interesting to optimize an extraction process to obtain maximum yields of these substances, useful as nutritional supports in the prevention of diverse diseases. There are many examples of the extraction of phenolic compounds from different fruits, leaves and woody plants (Dapke-

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vicius, Venskutonis, Van Beek, & Linssen, 1998; Lee, Mitchell, & Shibamoto, 2000; Peng, Lin, & Lin, 2000). For pine sawdust and almond hulls, several solvents and conditions have been used to carry out the extraction process. Siriwardhana and Shahidi (2002), for instance, have extracted phenolic compounds present in almond seed, skin and shell using 80% ethanol at 80 °C for 30 min. Kähkönen et al. (1999) used an 80% aqueous methanol solution for the extraction of the needle, cork and bark of the scotch pine in an ultra sound mixer for 1 min.

However, despite the abundant literature about this topic, very little is known about the influence of variables, such as temperature, time of contact and liquid-solid ratio, on the extraction of phenolic compounds with antioxidant properties. So, a study about the influence of these parameters on the extraction process in two quite different lignocellulosic materials—almond hulls and pine sawdust—is described in this work. Through an experimental design, subjected to a mathematical treatment, the goal is to establish the best conditions for obtaining extracts rich in polyphenolics, with high antioxidant capacity.

2. Materials and methods

2.1. Materials

Samples of pine (*Pinus pinaster*) were supplied by MANUEL BOUZAS GARRIDO, S.A. (Vedra, La Coruña, Spain) and almond hulls (*Prunus amygdalus*) by BORGES, S.A. (Tárrega, Lleida, Spain).

Pine was ground in a knife mill. A giratory mortar of agate was used to grind the almond samples. Both powdered samples were sieved to select particles smaller than 0.5 mm and stored at room temperature until used.

2.2. Humidity

Samples were maintained in a stove at 105–108 °C until they reached constant weight. The results are the averages of three samples. Humidities were, for pine sawdust 28.77±2.74% and for almond hulls 10.00±0.03%. Throughout this work all the results are expressed on a dry basis.

2.3. Solvent extraction

The samples were subjected to extraction in a rotatory shaker G24 New Brunswick Scientific Co. Inc. (N.J., USA) at constant stirring rate of 120 rpm. The process was carried out with the following solvents: methanol and 96% ethanol (DROGAS VIGO, S.L., Porriño, Spain) and distilled water acidified with HCl (pH = 4). Solids were separated by filtration and the extracts were analyzed.

2.4. Experimental design

A full factorial 2^3 experimental design was developed to evaluate the effect of the temperature (**T**), time of contact (**t**) and liquid–solid ratio (**L/S**) (Box, Hunter, & Hunter, 1999). Temperature values varied between 25 and 50 °C, time contact between 30 and 90 min and liquid:solid ratio between 5:1 and 10:1. Variables were codified in the way that their value ranged between +1 and −1, taking, as the central point, the zero value. So,

$$t = (\mathbf{t} - 60)/30$$

$$T = (\mathbf{T} - 37.5)/12.5$$

$$L/S = (\mathbf{L/S} - 7.5)/2.5$$

Table 1 shows the factorial design matrix, with variables in both coded/non-coded form, for better comprehension. Numbers 1–12 corresponding with those in Tables 2–4.

Data were adjusted to a response surface R:

$$R = a_0 + a_1\ t + a_2\ T + a_3\ L/S + a_{12}\ tT + a_{13}\ tL/S + a_{23}\ TL/S + a_{123}\ tTL/S$$

Where a_0 is the value of the objective function in the central point conditions, a_1, a_2, a_3 represent the principal effects associated to each variable and the other ones represent the crossed effects among variables.

2.5. Determination of extractables

The determination of total solids was carried out after evaporating the solvent in a Büchi Rotavapor R-114. Maximum value of total extractables for each material was previously obtained in a Soxhlet extractor during 8 h. Results are shown in Fig. 1.

Table 1
Extraction conditions of the experimental design. Not coded/coded variables

Experiment	t (min)	T (°C)	L/S	t (min)	T (°C)	L/S
1	30	25	10	−1	−1	1
2	30	50	10	−1	1	1
3	30	25	5	−1	−1	−1
4	30	50	5	−1	1	−1
5	90	25	10	1	−1	1
6	90	50	10	1	1	1
7	90	25	5	1	−1	−1
8	90	50	5	1	1	−1
9	60	37.5	7.5	0	0	0
10	60	37.5	7.5	0	0	0
11	60	37.5	7.5	0	0	0
12	60	37.5	7.5	0	0	0

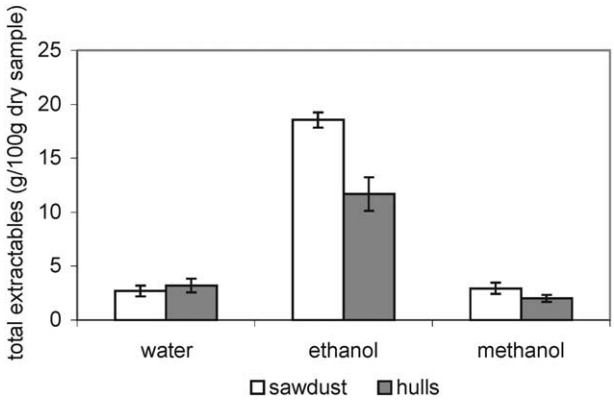

Fig. 1. Maximum extractables, after 8 h in a Soxhlet extractor, for pine sawdust and almond hull.

2.6. Determination of total polyphenolic compounds

The total phenolics were assayed colorimetrically by means of the Folin-Ciocalteu method, as modified by Singleton and Rossi (1965). 2.5 ml of ten-fold diluted Folin-Ciocalteu reagent, 2 ml of 7.5% sodium carbonate, and 0.5 ml of phenolic extract were mixed well. The absorbance was measured at 765 nm after 15 min heating at 45 °C. A mixture of water and reagents was used as a blank. The content of phenolics was expressed as gallic acid equivalents.

2.7. Determination of the antioxidant capacity

A DPPH radical-scavenging assay was performed using the method described by Von Gadow, Joubert, and Hansmann (1997) to determine the hydrogen-donating ability of the crude extract. A volume of 1.85 ml of 6.1×10^{-5} M. DPPH· methanol solution was used. The reaction was started by the addition of 150 μl of sample. The bleaching of DPPH· was followed at 515 nm (Shimadzu UV-160A) at 25 °C for 16 min. The inhibition percentage (IP) of the DPPH· radical was calculated as follows:

$$IP = \frac{(\text{absorbance}_{t=0\text{min}} - \text{absorbance}_{t=16\ \text{min}})}{(\text{absorbance}_{t=0\ \text{min}})} \times 100$$

2.8. Statistical analysis

The results reported in this work are the averages of at least three measurements, and the coefficients of variations, expressed as the percentage ratio between standard deviations (SD) and the mean values, were found to be <10 in all cases. Significant variables were calculated, subjecting results to a linear regression, using SPSS statistical programme version 10.0 (SPSS Inc., Chicago, Illinois). Only variables with a confidence level superior to 95% ($P<0.05$) were considered as significant.

3. Results and discussion

3.1. Total extractables

Fig. 1 shows the results of the characterization of materials in the three solvents. Ethanol was the best extractor agent for both substrates in comparison to the other solvents—acidified water and methanol—whose maximum values of extractables were similar. When samples were subjected to the extraction conditions of the experimental design (Table 2), ethanol also offered the best results for extractables. However, when comparing Table 2 with Fig. 1, it can be seen that the ratio between total extracted solids and the maximum extractables was lower when ethanol was used as a solvent.

Table 2 shows that the two materials contained considerable amounts of extractable compounds. All the solvents had a clear ability to extract substances from these residues; lower results were obtained when using water as extractant. This perhaps was expected, since water is not a good solvent for phenolics (Julkunen-Tiito, 1985).

With regard to the conditions of the experimental design, the best results for extractable substances were in general obtained for the highest liquid-solid ratio, basically for water and ethanol. This is obvious, since diffusivity values increase with increasing differences of phenol concentration between the solid interface and the bulk of the liquid.

Analysing the results for total extractables, it could be deduced that optimal conditions for extraction were obtained for both pine and almond when ethanol was used as a solvent and the liquid-solid ratios were maximum. In the response function models for extractable percentage in the best solvent, a clear dependence with only this parameter was found, whereas time of contact (t) and temperature (T) were not significant.

$$\%\text{Extractables}_{\text{sawdust}} = 6.743 + 1.392\ L/S$$
$$F_{\text{mod}} = 110.78\ P < 0.000\ R^2 = 0.930$$

$$\%\text{Extractables}_{\text{almond}} = 2.423 + 1.103\ L/S$$
$$F_{\text{mod}} = 34.99\ P < 0.000 R^2 = 0.814$$

Extractable percentage from pine sawdust was higher than from almond hulls, although the latter could offer better results when finer ground (<0.5 mm). Similar results were obtained by Moure, Franco, Dominguez, Nuñez, and Lema (2000) in *Guevina avellana* hulls, for which ethanol extracts contained 1.93% of total soluble solids. Kähkonen et al. (1999) reported similar results to ours in extractables from Scotch pine bark (*Pinus sylvestris*).

Table 2
Percentage of total extractable compounds from pine sawdust and almond hull samples subjected to the extraction conditions of the experimental design[a]

No. experiment	Pine sawdust			Almond hull		
	Ethanol	Methanol	Water	Ethanol	Methanol	Water
1	7.88±0.67	2.41±0.00	1.21±0.04	3.78±0.16	0.41±0.03	0.77±0.2
2	8.19±0.06	2.86±0.18	1.22±0.04	3.45±0.11	0.6±0.00	0.85±0.09
3	4.59±0.31	2.84±0.25	0.95±0.1	1.34±0.00	0.35±0.01	0.25±0.02
4	5.23±0.27	2.58±0.12	1.04±0.03	1.17±0.04	0.56±0.04	0.26±0.05
5	7.85±0.14	2.68±0.00	1.11±0.09	4.46±0.11	0.57±0.04	1.07±0.08
6	7.90±0.69	2.71±0.09	1.30±0.01	2.94±0.08	0.87±0.1	0.92±0.07
7	5.47±0.38	2.52±0.2	0.70±0.23	1.87±0.06	0.46±0.01	0.31±0.05
8	5.39±0.23	2.68±0.03	1.32±0.01	1.43±0.00	0.77±0.06	0.55±0.1
9	7.29	2.7	1.65	1.80	0.44	0.7
10	7.08	2.7	1.86	1.79	0.5	0.8
11	7.12	2.76	1.75	2.04	0.4	0.75
12	6.92	2.75	1.85	1.75	0.49	0.79

[a] Percentage of total soluble solids with respect to material weight on dry basis. In all cases, 10 g of material was used to carry out the extraction.

Table 3
Percentage of total phenolic compounds from pine sawdust and almond hull samples subjected to the extraction conditions of an experimental design[a,b]

No. experiment	Pine sawdust			Almond hull		
	Ethanol	Methanol	Water	Ethanol	Methanol	Water
1	5.12±0.09	11.2±0.61	1.04±0.04	2.31±0.05	1.06±0.01	0.77±0.02
2	7.33±0.12	11.2±0.44	1.58±0.04	7.21±0.50	2.26±0.01	1.28±0.16
3	6.65±0.16	9.6±0.06	1.12±0.1	2.87±0.20	1.20±0.01	1.00±0.06
4	6.47±0.09	8.55±0.08	1.44±0.13	5.50±0.13	2.07±0.02	1.06±0.03
5	6.37±0.28	8.65±0.53	1.36±0.09	3.20±0.07	1.36±0.08	1.01±0.03
6	7.86±0.09	10.73±0.08	1.94±0.01	4.58±0.04	4.12±0.07	1.78±0.05
7	8.22±0.6	9.35±0.52	1.53±0.03	3.78±0.2	1.47±0.07	0.80±0.01
8	7.45±0.3	9.55±0.08	1.88±0.01	6.1±0.02	3.01±0.00	1.26±0.03
9	7.51	9.68	1.40	2.52	2.14	1.05
10	7.49	10.01	1.32	3.04	2.19	1.05
11	7.93	9.41	1.36	2.94	2.16	1.05
12	7.17	10.30	1.36	2.92	2.24	1.09

[a] Percentage of total polyphenolics with respect to material weight on dry basis.
[b] All results are expressed as percentage of total phenolic compounds×100.

Table 4
Effects of extracts on DPPH free radicals (expressed as a percentage of inhibition of direct extract)

No. experiment	Pine sawdust			Almond hull		
	Ethanol	Methanol	Water[a]	Ethanol	Methanol	Water
1	10.42±3.25	23.66 ±1.63	–	2.15±0.28	14.92±2.89	3.15±0.12
2	17.04±5.28	19.41±2.82	–	6.14±1.42	18.22±2.67	7.89±1.99
3	24.56±0.04	25.82±2.02	–	10.97±0.21	37.01±0.73	16.07±3.29
4	26.00±8.68	30.62±3.28	–	17.64±0.21	47.94±2.17	27.27±1.54
5	15.57±2.97	19.02±1.16	–	3.19±0.28	16.67±0.25	5.65±1.82
6	21.14±0.61	11.61±1.26	–	8.92±0.02	27.15±2.53	12.53±0.34
7	25.84±0.45	28.71±3.24	–	15.32±0.7	37.23±2.63	15.36±1.59
8	31.85±4.36	34.17±0.04	–	36.21±0.58	58.05±0.96	27.75±2.12
9	27.30	18.25	–	10.07	28.94	9.68
10	15.75	19.77	–	10.14	28.94	14.21
11	16.90	16.80	–	13.13	29.16	11.09
12	17.06	16.77	–	12.09	30.46	13.94

[a] All the extracts of pine sawdust in water showed a negligible percentage of inhibition on DPPH free radicals, in all cases lower than 5%.

3.2. Phenolic compounds

Table 3 shows the yields of phenolic compounds. Methanol was the extractant with best results for phenols from pine sawdust. In almond hulls, however, ethanol was the best extraction solvent. In both materials, the polyphenols:extractables ratio in methanol, was about twice higher than those of ethanol and water extracts.

The best value for pine sawdust was 0.1122% in methanol, results in ethanol being about 30% lower. Values of total polyphenols in other tree extracts are numerous. Wood et al. (2002) reported polyphenol values of 0.35 g/100 g for extracts of *Pinus radiata* bark in distilled water, showing that bark is much richer in polyphenolic compounds that other woody parts.

Applying statistical analysis to the polyphenol concentration values of pine sawdust extracts, significant models for all solvents were obtained:

$$\%\text{Polyphenols}_{\text{ethanol}} \times 10^2 = 7.13 + 0.54\ t + 0.58TL/S$$
$$F_{\text{mod}} = 7.53\ P < 0.012\ R^2 = 0.656$$

$$\%\text{Polyphenols}_{\text{methanol}} \times 10^2 = 9.86 + 0.59\ L/S + 0.42tT\text{-}0.47tL/S + 0.36TL/S$$
$$F_{\text{mod}} = 8.71\ P < 0.008\ R^2 = 0.832$$

$$\%\text{Polyphenols}_{\text{water}} \times 10^2 = 1.44 + 0.19\ t + 0.22T$$
$$F_{\text{mod}} = 41.28\ P < 0.000\ R_2 = 0.902$$

In almond hull extracts, the yield of phenolic compounds (0.0106–0.0412 g/100 g residue) was similar to those obtained from other agricultural residues. Xing and White (1997), for instance, reported 0.056 g of total phenolic compounds/100 g solid in methanol extracts of oat hulls at 25 °C for 24 h. With water as solvent, Kumazawa, Taniguchi, Suzuki, Shimura, Kwon, and Nayakama (2002) carried out the extraction process on carob pods with boiling water for 10 min, obtaining 0.0192 g of total polyphenols/100 g, which accords with our study (0.0178 g of total polyphenols/100 g).

However, polyphenol concentrations are not always so low in natural products. Velioglu, Mazza, Gao, and Oomah (1998) studied the polyphenol content of several plant products, and found high values in buckwheat hulls (3.9 g of phenolic compounds/100 g), while phenol concentrations of $1.03.10^{-3}$–$4.23.10^{-3}$ g/100 g were found in *G. avellana* hull extracts (Moure et al., 2000).

For almond hull extracts, the response equations for the yields of polyphenolics are:

$$\%\text{Polyphenols}_{\text{ethanol}} \times 10^2 = 3.91 + 1.4T$$
$$F_{\text{mod}} = 34.99\ P < 0.000\ R^2 = 0.675$$

$$\%\text{Polyphenols}_{\text{methanol}} \times 10^2 = 2.11 + 0.42\ t + 0.8T + 0.13L/S + 0.28tT + 0.12tL/S + 0.19TL/S + 0.11tTL/S$$
$$F_{\text{mod}} = 110.41\ P < 0.000\ R^2 = 0.956$$

$$\%\text{Polyphenols}_{\text{water}} \times 10^2 = 1.1 + 0.093t + 0.23T + 0.09L/S + 0.083tT + 0.093tL/S + 0.095TL/S$$
$$F_{\text{mod}} = 46.1\ P < 0.000\ R^2 = 0.942$$

Clearly, significant variables in the extraction processes strongly depend on the solvent used and the material subjected to extraction. Except for ethanol, the complexity of the models is considerable, since they include many significant effects (even mixing effects). Chemical characteristics of the solvent and the diverse structure and composition of the natural products ensure that each material-solvent system shows different behaviour, which cannot be predicted.

In fact, there are many reports of different values for total polyphenols in materials that initially seem similar: aqueous methanol extracts of silver birch bark (*Betula pendula*) contained sixteen times less total phenolics (2.0±0.1 mg of GAE/g dry weight) than aspen bark (*Populus tremula*) (32.1±0.2 mg/g), which in turn had half the amount of phenolics of scotch pine bark (*Pinus sylvestris*) (76.0±2.9 mg/g) (Kähkönen et al., 1999). Matthäus (2002) used different mixtures of organic solvents to carry out the extraction of residues of different oilseeds. No trend was found for the contents of the extracts.

Fig. 2 shows the surface response plot for the polyphenol content when methanol was used for the extraction process with pine sawdust. Plots are made to 30 and 90 min of contact time. This indicates that an increase of the contact time minimizes the effect of the other parameters in the extraction of pine sawdust.

Finally, no correlation was found between the solubles extraction yield and the polyphenolic content. Both the lignocellulosic materials and the extraction solvent influenced the amount of total phenolic compounds.

3.3. Antioxidant activities

Table 4 shows the percent inhibition of the H· radical for the studied pine sawdust and almond hull extracts. It was calculated from the decrease in absorbance of the DPPH· radical caused by antioxidants, due to the scavenging of the radical by hydrogen donation. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The DPPH· method has

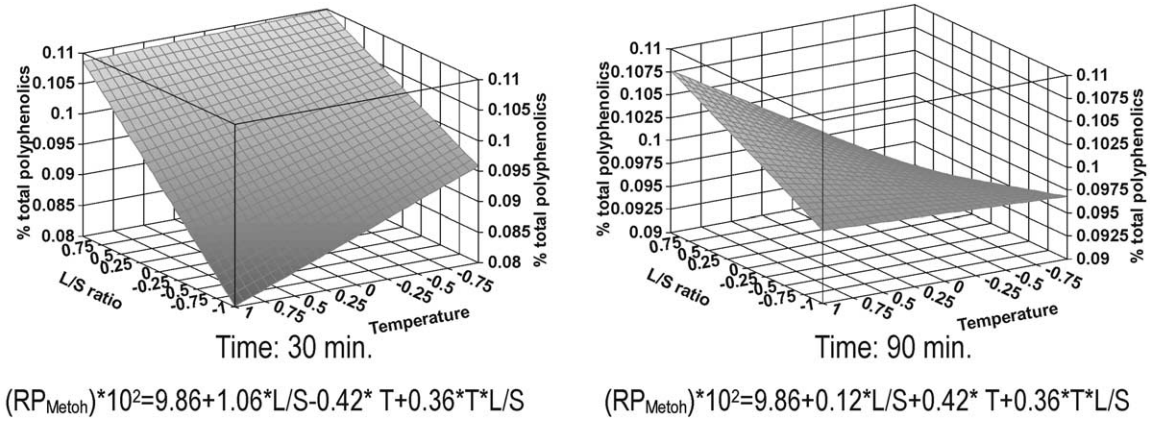

Fig. 2. Response surface plot for percentage of polyphenols in extracts of pine sawdust in methanol.

been widely used to measure the antioxidant capacities of different residual and natural products, it being a rapid, simple, sensitive and practical assay (Siriwardhana & Shahida 2002; Moure et al., 2000; Peng, Lin, & Lin, 2000).

In this case, best results were obtained under the same operational conditions for all cases (except experiment no. 8): higher time contact (90 min), higher temperature (50 °C) and lower liquid–solid ratio (5:1).

Methanolic extracts had the best antioxidant activity on the DPPH radicals although, for extracts of pine sawdust, values in ethanol were very close; however, in almond hull extracts, the antioxidant activities with different solvents conspicuously obey the order methanol > ethanol > water. A similar trend was observed for the DPPH inhibition in other residual hulls (Moure et al., 2000).

Scarce literature exists about the comparison of radical-scavenging ability of methanolic and ethanolic extracts from the same material. However, it is not difficult to find reports for water-ethanolic extracts. Singh, Chiadambra, and Jayaprakasha (2002) have tested the antioxidant power of methanol and water extracts of pomegranate peel using the DPPH method. At 50 ppm of extract concentration, methanol and water extracts show 81 and 43% of inhibition, respectively. Mancini-Filho, Van-Koiij, Manzini, Cozzolino, and Torres (1998) also reported a higher antioxidant capacity of the alcoholic extract than the aqueous one in cinnamon extracts. This demonstrates the superior capacity of alcohol extracts to scavenge free radicals.

Significant variables change, depending on material and solvent used. Equations of response surface were:

(a) For Almond Hulls:

$$\%\mathrm{Inh}_{\mathrm{ethanol}} = 12.16 + 3.34\ t + 4.66T - 7.47L/S + 2.0tT - 2.39tL/S - 2.23TL/S$$
$$F_{\mathrm{model}} = 22.81\ P < 0.002\ R^2 = 0.893$$

$$\%\mathrm{Inh}_{\mathrm{methanol}} = 31.3 + 2.63\ t + 5.69T - 12.91L/S + 2.13tT - 2.25TL/S$$
$$F_{\mathrm{model}} = 105.12\ P < 0.000\ R^2 = 0.987$$

$$\%\mathrm{Inh}_{\mathrm{water}} = 13.72 + 4.40\ T - 7.15L/S$$
$$F_{\mathrm{model}} = 42.3\ P < 0.000\ R^2 = 0.903$$

(b) For Pine Sawdust:

$$\%\mathrm{Inh}_{\mathrm{ethanol}} = 20.79 - 5.51\ L/S$$
$$F_{\mathrm{model}} = 12.64\ P < 0.005\ R^2 = 0.817$$

$$\%\mathrm{Inh}_{\mathrm{methanol}} = 22.05 - 5.7\ L/S$$
$$F_{\mathrm{model}} = 11.74\ P < 0.006\ R^2 = 0.897$$

In general, results for antioxidant activity are low in comparison to those found in the literature for other hulls. Franco (2002), for instance, worked with Chilean hazelnut hulls, finding values of the percentage of inhibition of 85.2%, using methanol as a solvent, whereas in our hulls, the maximum value was 58.1%. Moure et al. (2000) reported 82% of inhibition for methanol extracts of *Guevina avellana* hulls.

4. Conclusions

The sawdust of *Pinus pinaster* and the hulls of *Prunus amygdalus* are lignocellulosic residues that contain antioxidant compounds. The extracts obtained from these materials have remarkable radical-scavenging activities (DPPH), whose values vary as a function of the different extraction conditions. The best results for the percentage of inhibition were reached using methanol as an extractant (58% and 34% for almond hulls and pine sawdust, respectively).

Many variables influence the yields of extractables and polyphenolics, the value of liquid–solid ratio for each solvent being decisive; temperature and time contact were also important. Factors to consider in future experiments, in order to increase the antioxidant activity, would be the particle size or the optimization of stirring.

Lastly, there was no correlation between concentrations of extractables, concentrations of polyphenols and antioxidant capacities. For this, characterization of individual phenolics, in order to study their contribution to total antioxidant capacity, would be interesting.

Acknowledgements

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References

Ames, B. M. (1983). Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science*, *221*, 1256–1264.

Box, G. E. P., Hunter, W. G., & Hunter, J. S. (1999). *Estadística para investigadores. Introducción al diseño de experimentos, análisis de datos y construcción de modelos*. Barcelona: Reverté, SA.

Cho, K., Yun, C., Ion, D., Cho, Y., Rimbach, G., Packer, L., & Chung, A. (2000). Effect of bioflavonoids extracted from the bark of *Pinus maritime* on proinflamatory cytokine interleukin-1 production in lipopolysaccharide-stimulated RAW 264.7. *Toxicology and Applied Pharmacology*, *168*, 64–71.

Cruess, W. V., Kilbuck, J. H., & Hahl, E. (1947). Utilization of almond hulls. *Fruit Products Journal*, *26*, 363–365.

Dapkevicius, A., Venskutonis, R., Van Beek, T., & Linssen, J. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Journal of the Science of Food and Agriculture*, *77*, 140–146.

Decharneux, T., Dubois, F., Beauloye, C., Warriaux-de Coninck, S., & Wattiaux, R. (1992). Effects of various flavonoids on lysosomes subjected to an oxidative or an osmotic stress. *Biochemical Pharmacoogy*, *44*, 1243–1248.

Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E., Willett, W. C., & Colditz, G. A. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute*, *92*, 1812–1823.

Franco, D. (2002). *Procesado de oleaginosas: extracción etanólica de avellana chilena y rosa mosqueta*. Universidad de Santiago de Compostela: Dpto. Ingeniería Química.

Gordon, M. H. (1996). Dietary antioxidants in disease prevention. *Natural Product Reports*, 265–273.

Julkunen-Tiito, R. (1985). Phenolic constituents in the leaves of northern willows, methods for the analysis of certain phenolics. *Journal of Agricultural & Food Chemistry*, *33*, 213–217.

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

Kumazaya, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M. S., & Nakayama, T. (2002). Antioxidant activity of polyphenols in carob pods. *Journal of Agricultural & Food Chemistry*, *50*, 373–377.

Lee, K., Mitchell, A., & Shibamoto, T. (2000). Determination of antioxidant properties of aroma extracts from various beans. *Journal of Agricultural & Food Chemistry*, *48*, 4817–4820.

Mancini-Filho, J., Van-Koiij, A., Manzini, D. A. P., Cozzolino, F. F., & Torres, R. P. (1998). Antioxidant activity of cinnamon (*Cinnamomum zeylanicum*, Breyne) extracts. *Bolletino Chimico Farmaceutico*, *137*, 443–447.

Matthäus, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agricultural & Food Chemistry*, *50*, 3449–3452.

Michels, K. B., Giovannucci, E., Joshipura, K. J., Rosner, B. A., Stampfer, M. J., Fuchs, C. S., Colditz, G. A., Speizer, F. E., & Willett, W. C. (2000). Prospective study of fruit and vegetable consumption on incidence of colon and rectal cancers. *Journal of the National Cancer Institute*, *92*, 1740–1752.

Moure, A., Franco, D., Sineiro, J., Domínguez, H., Núñez, M. J., & Lema, J. (2000). Evaluation of extracts from *Guevina avellana* hulls as antioxidants. *Journal of Agricultural & Food Chemistry*, *48*, 3890–3897.

Peng, C., Lin, Z., & Lin, G. (2000). Effect of light on scavenging capacity for organic free radical in leaves of four woody plants. *Zhiwu Xuebao*, *42*, 393–398.

Pietta, P., Simonetti, P., & Mauri, P. (1998). Antioxidant activity of selected medicinal plants. *Journal of Agricultural & Food Chemistry*, *46*, 4487–4890.

Rimbach, G., Virgili, F., Park, Y. C., & Packer, L. (1999). Effect of procyanidins from *Pinus maritime* on glutathione levels in endothelial cells challenged by 3-morpholinosydnonimine or activated macrophages. *Redox Report*, *4*, 171–177.

Shahidi, F. (2002).Phytochemicals of almond and their role in inhibition of DNA niching and human LDL cholesterol oxidation. In *Abstracts of papers, 224th ACS National Meeting, Boston, MA, USA*.

Singh, R. P., Chidambara, M., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural & Food Chemistry*, *50*, 81–86.

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

Siriwardhana, S., & Shahidi, F. (2002). Antiradical activity of extracts of almond and its by-products. *Journal of the American Oil Chemists' Society*, *79*, 903–908.

Steinberg, D. (1992). Metabolism of lipoproteins and their role in the pathogenesis of atherosclerosis. *Atherosclerosis Review*, *18*, 1–6.

Takeoka, G., & Dao, L. T. (2000) Antioxidant constituents of almond hulls. In *Abstracts Papers—American Chemical Society 2000, 220th AGDF-034*.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity of total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural & Food Chemistry*, *46*, 4113–4117.

von Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of the other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. *Journal of Agricultural & Food Chemistry*, *45*, 632–648.

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.

Xing, Y., & White, P. J. (1997). Identification and function of antioxidants from oat groats and hulls. *Journal of the American Oil Chemists' Society*, *74*, 303–307.