Evaluation of Extracts from *Gevuina avellana* Hulls as Antioxidants

Andrés Moure,† Daniel Franco,‡ Jorge Sineiro,‡ Herminia Domínguez,*,† María José Núñez,‡ and Juan M. Lema‡

Departamento de Enxeñería Química, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas 32004, Ourense, Spain, and Departamento de Enxeñería Química, Universidade de Santiago de Compostela, Avda. das Ciencias sn, 15706 Santiago de Compostela, Spain

The antioxidant activity of the extracts from *Gevuina avellana* hulls was evaluated and compared with that of BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), using the *β*-carotene bleaching assay, the accelerated oxidation of crude soybean oil, and the 2,2-diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging method. Solvents of different polarity were used to obtain the extracts. Both the extraction yield and the antioxidant activity were strongly dependent on the solvent. The ethanol and diethyl ether soluble fractions were the most active with the *β*-carotene assay. Ethanol and methanol extracts were the most active in hydrogen radical scavenging activity. Water and methanol inhibited more efficiently the oxidation of soybean oil at 70 and 80 °C, respectively. As a general trend, increased antioxidant activity was observed for increased extract concentration. Except the acetone extracts, all were stable after 6 months storage at 4 °C. The ethanol solubles from *G. avellana* hulls present antioxidant activity similar to that of synthetic antioxidants and to other reported residual agroindustrial materials.

Keywords: *Gevuina avellana; hulls; natural antioxidants; polyphenolics; solvent polarity*

INTRODUCTION

General public concern in regard to food additives and questions on the safety of synthetic antioxidants have led to the research of natural sources of food-grade antioxidants, especially among vegetable materials (plant leaves, seeds, barks, residues, and so on). Currently, byproducts of agro-industries are wasted or destined to undervalued uses, and they could be an economical and attractive source of natural antioxidants. The extraction of antioxidant compounds from residual materials such as hulls (Yen et al., 1993; Duh and Yen, 1995, 1997; Duh et al., 1997; Watanabe et al., 1997; Xing and White, 1997), seed coats (Muanza et al., 1998), peels (Larrauri et al., 1997; Rodríguez de Sotillo et al., 1994a,b), grape seeds (Gabrielska et al., 1997; Wulff, 1997; Pietta et al., 1998; Yamaguchi et al., 1999), pomace (Meyer et al., 1998; Saura-Calixto, 1998; Lu and Foo, 2000), olive rape (Sheabar and Neeman, 1988), olive mill wastewaters (Visioli et al., 1999), barks (Marinova et al., 1994), cocoa byproducts (Azizah et al., 1999), old tea leaves (Zandi and Gordon, 1999), carrot pulp waste (Chen and Tang, 1998), nonvolatile residue from orange essential oil (Vargas-Arispuro et al., 1998), and wheat bran (Baublis et al., 2000) has been reported. Hulls play the major role in the defense of the plant seeds and together with bran fractions concentrate most tannin; therefore, the antioxidant activity of these fractions is higher than that of seeds (Tsuda et al., 1994; Lehtinen and Laakso, 1998; Shahidi and Naczk, 1995).

Gevuina avellana is a native Chilean oilseed. The kernel contains 50% its dry weight as oil with excellent properties for food and cosmetics; the defatted meal of *G. avellana* contains 25% well-balanced protein, which is suitable as food ingredient. The hulls content accounts for 66−70% dry weight, with no reported use except for burning (3900 kcal/kg). Although still underexploited, this seed is a promising agricultural resource for the Andean area: both the oil and meal can be used for food purposes and the hulls could be a source of antioxidant compounds.

The yield and antioxidant activity of natural extracts is closely dependent on the solvent used for extraction (Julkunen-Tiito, 1985; Kim et al., 1994; Marinova and Yanishlieva, 1997; Muanza et al., 1998). This fact can be due mainly to the different polarity of the compounds with antioxidant activity and to the extraction of substances with either prooxidant action, such as chlorophyll, or antioxidant activity, such as dipeptides or polyphenolics (Amarowicz and Shahidi, 1997; Hattori et al., 1998; Kansci et al., 1997). The amount of phenolics extracted is affected by temperature and other operational factors such as the previous conditioning of the samples (Julkunen-Tiito, 1985; Larrauri et al., 1997; Meyer et al., 1998; Lehtinen and Laakso, 1998; Azizah et al., 1999; Zhishen et al., 1999). A factor also affecting polyphenolic content in plants is the maturity of the cultivars (Yen and Duh, 1995).

Oxidation in biological and in food products may involve free-radical scavenging, metal chelation, and decomposing peroxides, and often more than one mechanism is involved. The assays to measure the antioxidant activity usually involve these reactions. The antioxidant activity is very system-dependent, and according to the assays and the lipidic system used as substrate, a wide range of activities can be determined. Therefore, a single assay would not be representative of the antioxidant potential of a mixture of compounds because those contained in crude natural extracts and different

* Telephone: +34 981 653100 ext. 14224. Fax: + 34 981 595012. E-mail: eqmajose@usc.es.
† Universidade de Vigo.
‡ Universidade de Santiago de Compostela.

10.1021/jf000048w CCC: $19.00
Published on Web 08/12/2000

in vivo and in vitro tests could provide a more reliable approach to assess the antioxidant activity.

The aim of this work is to evaluate the antioxidant activity of *G. avellana* hulls. For this purpose, solvents with different polarity were used and compared with regard to (i) extraction yield of soluble solids and total phenolics, (ii) antioxidant activity with assays involving media with different polarity, and (iii) comparison of the antioxidant potential with that of other agroindustrial residues.

MATERIALS AND METHODS

Materials. *Gevuina avellana* seeds were supplied by Forestal Casino Ltda. (Santiago, Chile). The seeds were dried at 50 °C, manually dehulled, ground in a coffee grinder, sieved to select particles smaller than 0.6 mm, and stored at 4 °C until used. Freshly extracted crude soybean oil was kindly supplied by Moyresa (A Coruña, Spain). This oil did not contain any added antioxidants other than the natural ones. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were from Analema (Vigo, Spain).

Solvent Extraction. The powdered hull samples were defatted overnight in a rotary shaker at room temperature with hexane, using a solid/liquid ratio of 1:15 g/g. The defatted solids were air-dried and separated into fractions to be subjected to extraction with the following solvents: acetone, butanol, diethyl ether, distilled water acidified with HCl (pH = 4.3), 96% ethanol, ethyl acetate, and methanol. The solid/liquid ratio was 1:15 g/g. Extractions were carried out overnight at room temperature, except for acidified water (3 h at $T = 40$ °C), since water is not a good solvent for phenolics (Julkunen-Tiito, 1985). Solids were recovered by filtration and subjected to two additional extraction steps under the same conditions.

Preparation of Freeze-Dried Extracts. The extracts were filtered and vacuum evaporated to remove the organic solvents. Water was removed by freeze-drying to avoid affecting the antioxidant activity (Larrauri et al., 1997). Freeze-dried extracts were weighted to determine the extraction yield and stored at 4 °C because temperature and light exposure promote chemical reactions and consequent hydrolysis of phenolic acids (Rodríguez de Sotillo et al., 1994a; Friedman, 1997).

Analytical Methods. *Color of the Extracted Meals.* Color values L^*, a^*, and b^* of the solid samples after solvent extraction were measured with a Macbeth Color Eye 2180. The instrument was standardized against a white tile ($L^* = 95.768$, $a^* = -0.254$, $b^* = 1.576$).

UV−Vis Spectra. The powdered extracts were redissolved in methanol, and UV−visible spectra of the solutions were recorded in a Hitachi U-2000 spectrophotometer, with methanol as reference.

Determination of Total Extractable Phenolics (TEP). The amount of total extractable phenolics in the freeze-dried extracts (TEP_{FD}) was determined according to the spectrophotometric AOAC method (952.03, Folin−Denis method) and by direct spectrophotometric absorption at 330 nm (TEP_{ABS}) against a standard calibration curve with chlorogenic acid (Sigma Chem. Co) as standard.

Determination of the Antioxidant Activity. β-Carotene Bleaching Method. The spectrophotometric method of Miller (1971), based on the ability of the different extracts to decrease oxidative losses of β-carotene in a β-carotene/linoleic acid emulsion, was used. A concentrated solution was prepared from each freeze-dried extract in ethanol. This concentration depends on the solubility of the extract at room temperature and is shown in Table 2 as the maximum value for each extract. Progressively diluted solutions were used for determining the effect of the concentration. A 2.0 mg sample of crystalline β-carotene (Merck) was dissolved in 10 mL of chloroform. One milliliter of this solution was then pipetted into a round-bottomed flask, which contained 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. After removal of chloroform on a rotary evaporator, 50 mL of oxygenated, distilled water was added to the flask with vigorous stirring in an Ultraturrax TC50. A 5 mL aliquot of the aqueous emulsion formed was then pipetted into each of a series of tubes, which contained 0.2 mL of ethanolic antioxidant solution. A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after addition of the emulsion to the antioxidant solution. A control sample with distilled water instead of extract was also analyzed for antioxidant activity. The tubes were then stoppered and placed in a water bath at 50 °C. Subsequent readings were taken at regular intervals until the carotene had been decolorized (about 3 h). The antioxidant activity was measured by the AAC (antioxidant activity coefficient), which is an estimation of the relative oxidation in the presence of extracts with respect to the oxidation in their absence.

$$\mathrm{AAC} = \frac{(\text{absorbance of extract}_{120\ \mathrm{min}} - \text{absorbance of control}_{120\ \mathrm{min}})}{(\text{absorbance of control}_{0\ \mathrm{min}} - \text{absorbance of control}_{120\ \mathrm{min}})} \times 1000$$

α,α-Diphenyl-β-picrylhydrazyl (DPPH•) Radical Scavenging Method. The DDPH• radical scavenging method as described by von Gadow et al. (1997a) was used to determine the hydrogen-donating ability of the crude extract. A minor modification was the use of 2 mL of a 3.6×10^{-5} M methanolic solution of DPPH• (Fluka), which was added to 50 μL of a methanolic solution of the antioxidant. The decrease in absorbance at 515 nm was recorded in a Hitachi U-2000 spectrophotometer for 16 min. The inhibition percentage (IP) of the DPPH• radical was calculated as follows:

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

Oxidation of Soybean Oil. Freeze-dried extracts (0.8 mL) redissolved in methanol were mixed with 10 mL of oil into a 50 mL glass flask, thoroughly homogenized, and placed in an oven at 70 or at 80 °C. Higher temperature assays (≈100 °C) are unreliable because hydroperoxides decompose at high temperatures; therefore, the synthetic antioxidants could volatilize, thus presenting poor activity (von Gadow et al., 1997a). The peroxide value (PV) that can be used as indicator of the primary oxidation of oils (Sheabar and Neeman, 1988) was determined by the UNE-55.023 method. Inhibition of oil oxidation (IO) was expressed as follows:

$$\mathrm{IO}\ (\%) = 100 - \frac{(\text{peroxide value increase})_{\mathrm{sample}}}{(\text{peroxide value increase})_{\mathrm{control}}} \times 100$$

Chemical antioxidants, BHA and BHT, were also tested for antioxidant activity and used as reference standards.

All tests and analyses were run in duplicate or triplicate and averaged. The deviation from the mean at the 95% confidence level was employed for determining the differences in color samples. The results from the other determinations in this work were compared by a student *t*-test (two-tailed) for paired samples at a 95% significance level. The SPSS statistical package (SPSS Inc.) was employed for statistical analysis.

RESULTS

Effect of the Solvent on the Yield of Total Soluble Solids and Polyphenolics. The extraction yield of soluble substances, expressed as percent by weight of the hulls, and the total extractable polyphenolics, expressed as chlorogenic acid equivalents, are closely dependent on the solvent, as shown in Table 1. The colorimetric method for phenol determination, based on the use of the Folin−Denis reagent, is not specific for phenols: reducing compounds can interfere

Table 1. Extract Yield of Total Soluble Solids and Total Extractable Phenolics (TEP[a]) in the Extract and Color of the Solid Samples of *G. avellana* Hulls

solvent	total sol. solids (g/100 g)	TEP_{FD} (g/100 g)	TEP_{ABS} (g/100 g)	L^*	a^*	b^*
ground hulls				50.99^c	5.866^c	9.185^c
acid. water (pH = 4.3)	2.53	1.98×10^{-3c}	7.47^d	45.85^d	4.785^d	6.879^c
methanol	2.75	2.69×10^{-3c}	9.63^c	49.47^c	5.134^c	8.292^c
ethanol	1.93	4.23×10^{-3b}	77.0^b	47.09^c	4.916^c	7.535^c
acetone	1.45	1.03×10^{-3d}	7.06^d	52.91^b	6.433^b	10.945^b
diethyl ether	1.10	n.d.	3.01^e	49.75^c	5.609^c	8.377^c

[a] TEP_{FD}: total extractable polyphenols, determined as chlorogenic acid equivalents by Folin-Denis method (n.d.: not detected). TEP_{ABS}: total extractable polyphenols, determined as chlorogenic acid equivalents by absorbance at 330 nm. [b–e] Values with same letter were not significantly different from mean value at a 95% confidence level. (Student *t*-test with $p < 0.05$ and 95% confidence interval for the mean; see Materials and Methods).

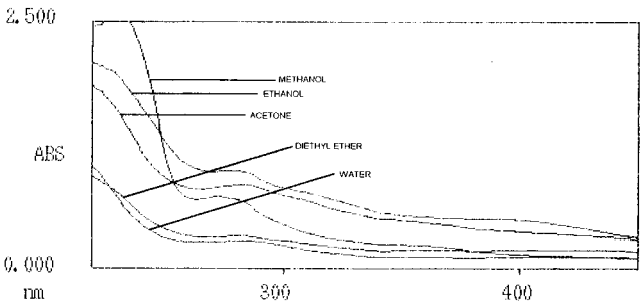

Figure 1. UV−visible spectra of the *G. avellana* hulls extracts at 0.1 g/L.

(Makkar, 1989), and its reactivity is different for different phenolics (Julkunen-Tiito, 1985). However, it is widely used and was chosen in order to obtain comparative results with other materials reported in the literature.

No correlation was found between the solubles extraction yield and the polyphenolics content. The highest solid extraction yield was obtained with methanol, followed by that obtained with water and with ethanol. Ethanol was the solvent which rendered more polyphenolic compounds, two times more than water and four times more than acetone. Ethyl acetate and butanol extracts were also prepared, but no polyphenolics were detected and the extract yield was negligible.

The color of solid residues after solvent extraction is shown in Table 1. Two samples were significantly different from others: that extracted with acidified water and that extracted with acetone. Extraction with acetone rendered a lighter solid residue (higher L^* values) and water a darker sample (lower L^* values). The residues from samples extracted with intermediate polarity solvents showed minor nonsignificant differences. The a^* and b^* color components in acetone and water extracts were also significantly different from those of other extracts.

The UV−visible spectra of the diluted extracts in methanol at 0.1 g/L concentration are shown in Figure 1. Absorption maxima were located under 225 nm, except for the methanolic extracts. Ethanol, methanol, and acetone extracts present smaller peaks absorbing in the range of 270−290 nm.

Antioxidant Activity and Effect of Extract Concentration. *β-Carotene Bleaching Inhibiting Activity.* The powdered extracts were redissolved in ethanol for measuring the antioxidant activity with the β-carotene bleaching method; the ethanolic extracts were obviously the most soluble in ethanol, followed by those extracted with diethyl ether and acetone. Figure 2 shows the decrease in absorbance of β-carotene for different concentration of extracts obtained with ethanol, methanol (Figure 2a), acidified water, diethyl ether (Figure 2b), and acetone (Figure 2c).

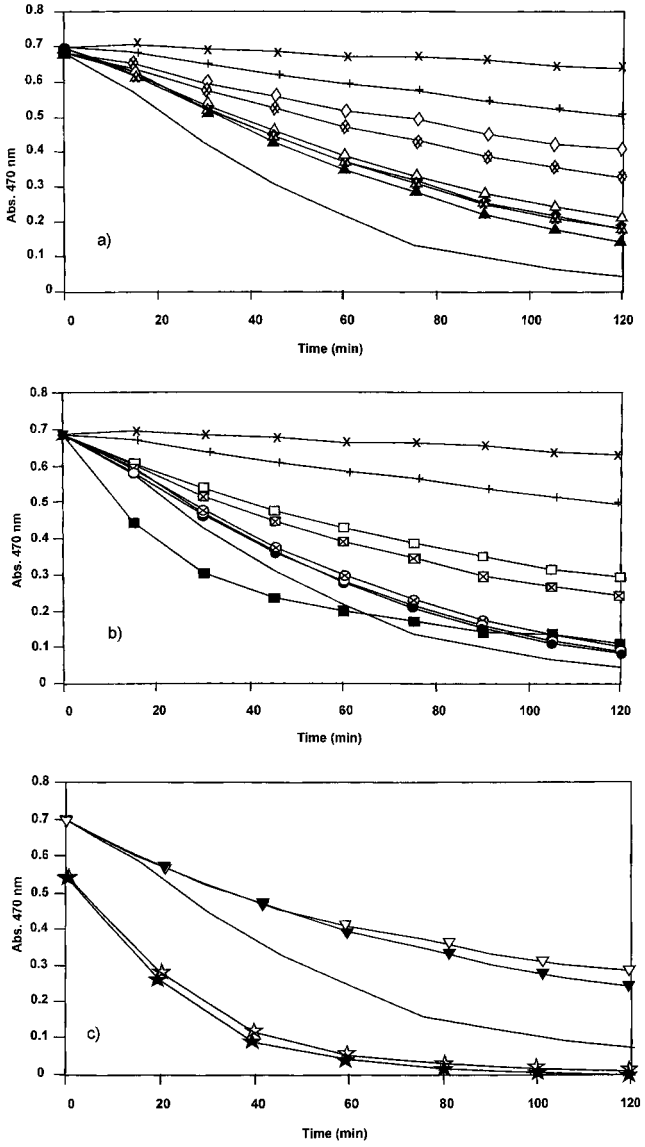

Figure 2. Decrease in absorbance at 470 nm of the extracts of *G. avellana* hulls assessed with the β-carotene/linoleic bleaching method: (—) control, (+) BHT, (×) BHA. (a) Ethanol extracts at 1100 (♦), 2200 (diamond with ×), and 4400 (◇) mg/L; methanol extracts at 410 (▲), 820 (triangle with ×), and 1100 (△) mg/L. (b) Water extracts at 600 (●), 1200 (⊗), and 1800 (○) mg/L; diethyl ether extracts at 715 (■), 1430 (square with x), and 2500 (□) mg/L. (c) Acetone extracts at 1600 (▼) and 2700 mg/L (▽) freshly prepared and at 1600 (★) and 2700 mg/L (☆) after 6 months at 4 °C.

Table 2. Antioxidant Activity of *G. avellana* Hulls Extracts at Different Concentration Measured by *β*-Carotene Bleaching Method (Mean ± SD)

extract	concn (mg/L)	AAC
acidified water	600	60.75 ± 3
	1200	96.57 ± 7
	1800	73.99 ± 3
methanol	410	36.80 ± 17
	820	119.91 ± 11[a]
	1100	183.98 ± 10
ethanol	1100	211.06 ± 8
	2200	506.23 ± 20
	4400	767.13 ± 18
acetone	400	−61.04 ± 13
	800	−2.16 ± 12
	1600	236.80 ±14
	2700	347.62 ± 27
diethyl ether	715	102.80 ±16[a]
	1430	326.32 ± 13
	2500	454.05 ± 12
BHA	230	901 ± 48
BHT	350	693 ± 30

[a] Values not significantly different at $p < 0.05$ (Student *t*-test).

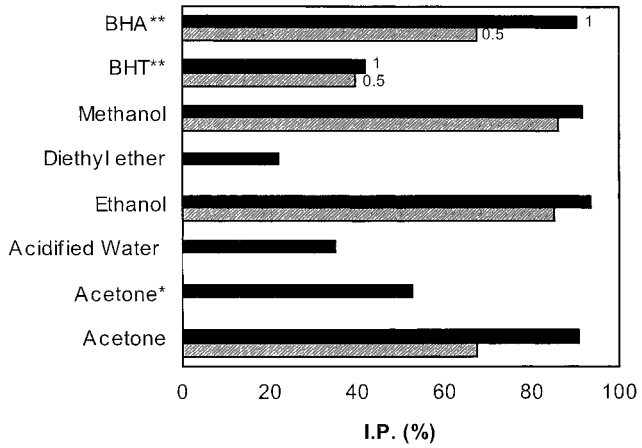

Figure 3. DPPH• inhibition by different extracts at (shaded bar) 500 and (■) 1000 mg/L concentration. Notes: *, measured after 6 months at 4 °C; **, "0.5" indicates 0.5 mol antioxidant/mol DPPH•, "1" indicates 1 mol of antioxidant/mol of DPPH•.

The calculated AAC values are summarized in Table 2. The highest concentration value shown in this table was the solubility of the freeze-dried extract in ethanol. The major antioxidant activities found for ethanol extract concentration of 4400 and 2200 mg/L were similar to the AAC values of BHT, but the concentration values of BHT 10 times lower.

Acetone extracts were found to have prooxidant action at concentrations under 1000 mg/L, and the lower the concentration, the higher the prooxidant effect. The absorbance of extracts with 400 and 800 mg/L was lower than that of the control at 120 min into the assay (data not shown). The lowest concentration of the diethyl ether extract tested (715 mg/L) showed prooxidant activity up to 70 min of reaction (see Figure 2b when compared to control) and thereafter a slight antioxidant activity.

DPPH• Radical Scavenging Activity. Figure 3 shows the percent of inhibition of the H• radical for the studied *G. avellana* hulls 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. It is visually noticeable as a discoloration from purple to yellow color. Ethanol and methanol extracts have shown similar DPPH• scavenging activity with a slight concentration effect. Acetone extracts showed similar antioxidant activity at 1 g/L but significantly lower activity at 0.5 g/L. This activity is higher than that measured for extracts obtained with less and with more polar solvents, such as diethyl ether and water, respectively.

Table 3. Inhibition of Soybean Oil Oxidation by Extracts of *Gevuina avellana*

extract	extract concn (g/L)	IO (%) after 19[†] 70 °C	IO (%) after 19[†] 80 °C	IO (%) after 40 h[†] 70 °C	IO (%) after 40 h[†] 80 °C
acidified water	1	86.92[b]	50.9[b]	77.1[b]	37.5[c]
methanol	1		73.13[a]		55.8[a]
ethanol	1	89.30[b]	9.5[e]	72.0[b]	−7.4[e]
acetone	1	98.7[a]	58.5[b]	74.0[b]	29.3[d]
diethyl ether	1	53.85[c]	43[d]	25.8[c]	47.2[b]
BHT	0.01	91.25[b]	34.78[c]	82.9[a]	34.3[c]
BHA	0.01	96.26[a]	12.8[e]	83.7[a]	55.8[a†]

[†] Values are percentage of inhibition oxidation (IO) as defined in Materials and Methods. Peroxide values in control samples increased from 1.8 to 35.4 and 38.7 mequiv/kg, at 70 and 80 °C, respectively. [a−e] Values with the same letter within a column are not significantly different at $p < 0.05$ (Student t-test).

Inhibition of the Lipid Oxidation. The course of oxidation for 40 h at 80 °C is presented in Figure 4. No oxidation was noticeable until 5−10 h exposure for any of the extracts. Then, it increased up to values ranging 32−78 mequiv/kg. Polymerization of oil was observed after 40 h. The extracts obtained with methanol, acetone, and diethyl ether retained antioxidant activity for prolonged oxidation periods. Inhibition of oxidation (IO) after 19 h exposure at 70 and at 80 °C is shown in Table 3. A dramatic decrease in antioxidant efficiency for ethanolic extracts caused by temperature can be observed. Some extracts, such as those in acetone and acidified water, retained at 80 °C more than 50% of the activity of that observed at 70 °C, the antioxidant effect being higher than that of diethyl ether and ethanol extracts. Except ethanol extracts, all of the extracts showed more thermal stability than BHT and BHA.

Stability of the Extracts. The color of the extracts ranged from light to dark yellow, and it was stable during storage for 6 months at 4 °C. The antioxidant action of these extracts was compared with that of freshly prepared extracts. No significant difference between freshly prepared and stored extracts ($p < 0.05$) was found by employing the *β*-carotene bleaching and DPPH• radical scavenging methods, except for the acetone extracts. These extracts lost 97% and 43% activity, respectively (Figure 2c; Table 3).

DISCUSSION

Solids extraction yield was around 1−2 g/100 of dried weight. The values obtained with polar solvents (water, methanol, ethanol, or their mixtures) from other residues can vary largely as a function of the employed material. It can be between 0.5 g/100 g solid for potato peel to values as high as 23.3 and 42 g/100 g solid found in lentil seeds and grape pomace, respectively (Muanza et al., 1998; Lu and Foo, 1999). The yield of phenolic compounds (TEP_{FD}) extracted from *G. avellana* hulls (1.03×10^{-3}−4.23×10^{-3} g/100 g residue) was lower than that obtained for other hulls and agricultural residue. Oat hulls contain 0.056 g/100 g solid of total phenolic compounds (Xing and White, 1997), and Lehtinen and Laakso (1998) reported 0.41 g/100 g phenolics from oat fiber. The measured polyphenolics content

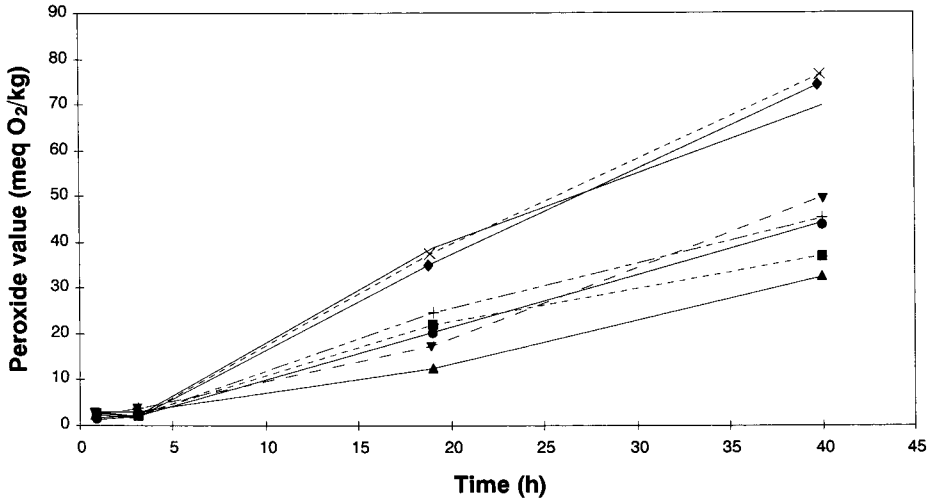

Figure 4. Oxidation of crude soybean oil treated with extracts of *G. avellana* hulls, BHA and BHT during storage at 80 °C: (−) control, (×) BHA, (♦) ethanol, (+) BHT, (■) diethyl ether, (●) water, (▼) acetone, (▲) methanol.

by this method must be taken carefully, as the sensitivity to low-molecular-weight polyphenolics is different from that to tannins, and different polyphenolics react in a different extension with the reactant (Makkar, 1989). Despite this, it is the most often employed method for tannins and polyphenolics. TEP_{ABS} show higher values that, although they could be affected by the presence of proteins, are in the range found for other residues: rape of olives (Sheabar and Neeman, 1988), lentil seeds coat (Muanza et al., 1998), grape pomace (Larrauri, et al., 1997), and buckwheat hulls (Watanabe et al., 1997).

The color of water extracts was slightly darker than that of other extracts. Although polyphenols oxidation is favored in alkaline media, it can occur in slightly acid media, such as what occurs during the well-known darkening of non-SO_2-treated white wines when exposed to air (which usually have a pH near 4.5). The concentration of metals in the water extract should be higher than that in less polar solvents, and it was reported that the presence of metals such as iron or manganese can catalyze the oxidation of polyphenols such as those present in olives (Brenes et al. 1995). The darkening of the water extract can be explained as being catalyzed by cations, such as iron, which are very common in plants.

As the polarity of the solvent increases, higher extraction yields of total soluble solids and total extractable polyphenolics were obtained. So, methanol and ethanol offer better results than acetone. Total extractable polyphenols obtained with ethanol were three more times than TEPs obtained with acetone. The literature offers very different results in this point: Wu et al. (1994) obtained results qualitatively similar to ours for wild rice hulls, whereas Julkunen-Tiito (1985), from northern willow leaves, obtained higher TEPs in the order 80% acetone, 80% methanol, distilled water, 0.2 M NaCl, and diethyl ether. Water is not a good solvent at the temperature of the present study (40 °C), this being a variable having a significant effect on extraction (Rodríguez de Sotillo et al., 1994a). But in our case, no limitation in extraction time is expected to occur at the employed conditions, so the total water-soluble material should have been extracted (von Gadow et al., 1997b).

The antioxidant activity of the extracts depends on the solvent, the concentration of the extract, and the antioxidant activity assay. The acute differences in activity are due to differences in the relative partition between phases in different apolar systems. The antioxidant activity assay in the β-carotene method showed as a main result that the higher the extract concentration, the higher the AAC value obtained. With this test, some prooxidant activity was detected (acetone extracts of up to 800 mg/L). This property has been observed for several antioxidants by other authors, as has been reported for caffeic acid (von Gadow et al., 1997a), which presented prooxidant activity with this assay and not in other assays. The extract concentration was a determinant factor of the prooxidant and antioxidant action. In this way, ginger and garlic extracts were reported to behave as prooxidants only at low concentration, in the presence of AEDT (deoxyribose assay) (Aruoma et al., 1997). BHT and BHA were assayed as standard antioxidants, the latter showing a better AAC using Miller's test. These results are in agreement with those of Karamac and Amarowicz (1997). Anyway, ethanol, diethyl ether, and acetone (>1600 mg/L) extracts rendered good AAC values. Moreover, the ethanol extracts were better than BHT in this assay.

Ethanol, methanol, and acetone extracts, used at 1 g/L concentration, had high antiradical activity measured as $DPPH^{\bullet}$ radical inhibition. It was always comparable to and higher than those of BHT and BHA (see Figure 3). The obtained $DPPH^{\bullet}$ inhibition levels with BHA and BHT were used as control for samples comparison and to validate the assay, being that these values were very close to those reported by von Gadow et al. (1997c). It was possible to confirm that *G. avellana* hulls extracts have the ability to enhance the stability against the primary oxidation. Diethyl ether extract shows a low activity at 70 °C, although its activity at 80 °C is highly preserved. Tsuda et al. (1994) found similar lipid peroxidation inhibition by both ethanol extracts from seed coats and methanol extracts, with ethyl acetate extracts being slightly more powerful antioxidants.

The extracts with the highest antioxidant activity (acetone, ethanol, methanol) were those with a high absorbance near 280 nm, which is probably derived from the presence of flavonoids. Absorbance near 330 nm was low, showing that phenolic acids content was not mainly responsible for the antioxidant activity. Only ethanol and acetone extracts showed a low or moderate absorbance at this wavelength. Since polyphenolic acids

showed a low proton donor activity (von Gadow et al., 1997a), other compounds must be the responsible for the observed antioxidant activity of the extracts. If polyphenolic acids were present in the ethanol extracts, being that the polyphenolic acids are highly soluble in ethanol, it would be expected that these extracts showed higher absorbance values. Moreover, total polyphenolics content, determined by the Folin−Denis method, was significantly greater in ethanol extracts (Table 1). The presence of flavonoids and not polyphenolic acids can explain these facts.

No correlation between DPPH• scavenging activity and inhibition of soybean peroxidation could be found. To have a high donating capacity is not the main factor to avoid radical generation in oil, which leads to peroxidation. The antioxidant activity in a β-carotene bleaching system was also different than the DPPH• radical scavenging activity. This latter was reported to increase with flavonoids concentration (von Gadow et al., 1997c). As a general trend, antioxidant activity increases with solvent polarity up to ethanol (activity in water < methanol < ethanol) and then decreases but not with a common trend for all extracts. Also, less polar solvents, such as acetone, gave good results in the inhibition of oil peroxidation and DPPH• radical scavenging. This behavior did not allow us to find statistically significant correlation factor for all of the extracts by several parametric and nonparametric statistical methods for testing correlation and covariance between populations. The extracts that showed a high antioxidant activity, such as DPPH• and β-carotene oxidation inhibition, were those exhibiting a peak between 300 and 250 nm and a maximum absorbance below 250 nm.

In general, the higher polyphenols extraction yield corresponds with higher antioxidant activity, probably due to the combined action of the present substances. The additive or synergistic effects of polyphenols makes the antioxidant activity of the crude extracts higher than that of isolated compounds or simulated extract. In experiments on sunflower oil oxidation, Rodríguez de Sotillo et al. (1994b) reported an antioxidant activity of the potato peel freeze-dried extract higher than that of a synthetic mixture of individual compounds, during sunflower oil oxidation. The main reason is that other substances could also act synergistically with the polyphenolics (Onyeneho and Hettiarachchy, 1992; Ogata et al., 1997). The valuable use of antioxidant compounds mixtures has been pointed out, since no single antioxidant performs optimally for all lipid products, and their combined use results in a synergistic effect (Yi et al., 1991). However, Watanabe et al. (1997) reported significantly higher peroxyl-radical scavenging activity for some of the purified fractions of buckwheat hulls extracts over those of the crude extracts.

Freeze-drying, as expected, is a suitable technique for preserving the antioxidant activity of any of the prepared extracts for prolonged periods (Rodríguez de Sotillo et al., 1994b). The extracts redissolved in ethanol and stored at 4 °C in the dark were stable after 6 months, except the acetone-soluble ones, which lost almost half of their radical scavenging activity and almost completely lost the capacity to inhibit the β-carotene oxidation.

This technique avoids exposure to high temperatures, which could cause a reduction in the polyphenolics content and antioxidant activity, as reported by several studies dealing with the effect of temperature during extraction or storage (Julkunen-Tiito, 1985; Larrauri et al., 1997; Zhishen et al., 1999).

Antioxidant Potential of *G. avellana* Hulls. The results obtained in this work indicate that *G. avellana* hulls are a potential source of natural antioxidants since the antioxidant activity of the extracts is comparable to those of extracts from other residual materials. The main drawbacks of this material are its relatively low and localized production, but it is cheap and accessible for the producing areas and could be used as stabilizer for oils and emulsions.

As examples, we can mention the activity of other seed hulls extracts, such as those reported by Duh and Yen (1997), who observed that methanolic extracts of peanut hulls (used at 0.48 and 1.20%) inhibited peroxidation of both soybean and peanut oils more efficiently than did BHA used at 0.02%. The peroxide value of soybean oil stored at 60 °C was similar in the presence of methanol extracts of oat hulls at 0.2 and 0.3% and when *tert*-butylhydroquinone (TBHQ) at 0.02% were used (Xing and White, 1997). Duh et al. (1997) reported lower TBARS (thiobarbituric acid-reactive substances) during accelerated oxidation of soybean oil at 60 °C in the presence of mung bean hulls (100 ppm) than with the same concentration of BHA or tocopherol after 10 days of storage. The peroxide value of soybean oil was significantly reduced with respect to control and was similar to samples treated with α-tocopherol or with BHA.

Gabrielska et al. (1997) observed 88 and 97% antioxidant activity of BHT for rose hips and grape seeds extracts, respectively, when both were extracted with ethyl acetate and precipitated with chloroform. The activity was measured as the percent of inhibition of egg yolk lecithin liposome oxidation. The linoleic acid oxidation inhibition and the hydrogen radical scavenging capacity of 1 g was similar to those found with 40 and 10%, respectively, of DL-α-tocopherol. Meyer et al. (1998) observed that grape pomace antioxidants presented 56% of the antioxidant activity presented by catechin during in vitro LDL oxidation.

Further investigation of in vitro and in vivo antioxidant activity of *Gevuina avellana* hulls, including (i) an assessment of the toxicity and the functionality of these extracts in food systems and (ii) the identification of the different compounds and the contribution of each component to the overall antioxidant action, are underway.

LITERATURE CITED

AOAC. Method 952.03. *Official Methods of Analysis of AOAC International*, 16th ed., 3rd. rev.; Cunniff, P., Ed.; AOAC International: Gaithersburg, MD, 1997.

Amarowicz, R.; Shahidi, F. Antioxidant activity of peptide fractions of capelin protein hydrolysates. *Food Chem*. **1997**, *58*, 355−359.

Aruoma, O. I.; Spencer, J. P. E.; Warren, D.; Jenner, P.; Butler, J.; Halliwell, B. Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem*. **1997**, *60*, 149−156.

Azizah, A. H.; Ruslawati, N. M.; Swee Tee, T. Extraction and characterization of antioxidant from cocoa byproducts. *Food Chem*. **1999**, *64*, 199−202.

Baublis, A.; Decker, E. A.; Clydesdale, F. M. Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. *Food Chem*. **2000**, *68*, 1−6.

Brenes, M.; Romero, C.; García, P.; Fernández, A. G. Catalytic effect of metal cations in the darkening reactions in ripe olive processing. *Z. Lebensm.-Unters.-Forsch*. **1995**, *201*, 221−225.

Chen, B. H.; Tang, Y. C. Processing and stability of carotenoid powder from carrot pulp waste. *J. Agric. Food Chem*. **1998**, *46*, 2312−2318.

Duh, P.-D.; Yen G−C. Changes in antioxidant activity and components of methanolic extracts of peanut hulls irradiated with ultraviolet light. *Food Chem*. **1995**, *54*, 127−131.

Duh, P.-D.; Yen G−C. Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. *J. Am. Oil Chem. Soc*. **1997**, *74*, 745−748.

Duh, P.-D.; Yen W. J.; Du, P.-C.; Yen. G−C. Antioxidant activity of mung bean hulls. *J. Am. Oil Chem. Soc*. **1997**, *74*, 1059−1063.

Friedman, M. Chemistry, biochemistry, dietary role of potato polyphenols. *J. Agric. Food Chem*. **1997**, *45*, 1523−1540.

Gabrielska, J.; Oszmianski, J.; Lamer-Zarawska, E. Protective effect of plant flavonoids on the oxidation of lecithin liposomes. *Pharmazie* **1997**, *52*, 2−3.

Hattori, M.; Yamaji-T.; K.; Kimagai, H.; Feng, Y.; Takahashi, K. Antioxidative activity of soluble elastine peptides. *J. Agric. Food Chem*. **1998**, *46*, 2167−2170.

Julkunen-Tiito, R. Phenolic constituents in the leaves of northern willows, methods for the analysis of certain phenolics. *J. Agric. Food Chem*. **1985**, *33*, 213-217.

Kansci, G.; Genot, C.; Meynier, A.; Gandemer, G. The antioxidant activity of sine and its consequences on the volatile profiles of liposomes during iron/ascorbate induced phospholipid oxidation. *Food Chem*. **1997**, *60*, 165−175.

Karamac, M.; Amarowicz, R. Antioxidant activity of BHA, BHT and TBHQ examined with Miller's test. *Grasas Aceites* **1997**, *48*, 83−86.

Kim, S. Y.; Kim, J. H.; Kim, S. K.; Oh, M. J.; Jung, M. Y. Antioxidant activity of selected oriental herb extracts. *J. Am. Oil Chem. Soc*. **1994**, *71*, 633−640.

Larrauri, J. A.; Rupérez, P.; Saura-Calixto, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *J. Agric. Food Chem*. **1997**, *44*, 1390−1393.

Lehtinen, P.; Laakso, S. Effect of extraction conditions on the recovery and potency of antioxidants in oat fiber. *J. Agric. Food Chem*. **1998**, *46*, 4842−4845.

Lu, Y.; Foo, L. Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem*. **2000**, *68*, 81−85.

Lu, Y.; Foo, L. Y. The polyphenol constituents of grape pomace. *Food Chem*. **1999**, *65*, 1−8.

Makkar, H. P. Protein precipitation methods for quantitation of tannins: a review. *J. Agric. Food Chem*. **1989**, *37*, 1197−1202.

Marinova, E. M.; Yanishlieva, N. V.; Kostova, I. N. Antioxidative action of the ethanolic extract and some hydroxycoumarins of *Fraxinus ornus* bark. *Food Chem*. **1994**, *51*, 125−132.

Marinova, E. M.; Yanishlieva, N. V. Antioxidative activity of extracts from selected species of the family *Lamiaceae* in sunflower oil. *Food Chem*. **1997**, *58*, 245−248.

Meyer, A. S.; Jepsen, S. M.; Sörensen, N. S. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. *J. Agric. Food Chem*. **1998**, *46*, 2439−2446.

Miller, H. E. A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc.* **1971**, *45*, 91.

Muanza, D.; Robert, R.; Sparks, W. Antioxidant derived from lentil and its preparation and uses. U.S. Patent US5762936, 1998.

Ogata, M.; Hoshi, M.; Shimotohno, K.; Urano, S.; Endo, T. Antioxidant activity of magnolol, honokiol and related phenolic compounds. *J. Am. Oil Chem. Soc.* **1997**, *74*, 557−568.

Onyeneho, S. N.; Hettiarachchy, N. S. Antioxidant activity of durum wheat bran. *J. Agric. Food Chem*. **1992**, *40*, 1496−1500.

Pietta, P.; Simonetti, P.; Mauri, P. Antioxidant activity of selected medicinal plants. *J. Agric. Food Chem*. **1998**, *46*, 4487−4490.

Rodríguez de Sotillo, D.; Hadley, M.; Holm, E. T. Phenolics in aqueous potato peel extract, extraction, identification and degradation. *J. Food Sci*. **1994a**, *59*, 649−651.

Rodríguez de Sotillo, D.; Hadley, M.; Holm, E. T. Potato peel waste, stability and antioxidant activity of a freeze-dried extract. *J. Food Sci*. **1994b**, *59*, 1031−1033.

Saura-Calixto, F. Antioxidant dietary fiber product: a new concept and a potential food ingredient. *J. Agric. Food Chem*. **1998**, *46*, 4303−4312.

Sheabar, F. Z.; Neeman, I. Separation and concentration of natural antioxidants from the rape of olives. *J. Am. Oil Chem. Soc.* **1988**, *65*, 990−993.

Shahidi, F.; Naczk, M. Phenolic compounds in cereals and legumes. In *Food Phenolics. Sources, Chemistry, Effects and Applications*; Technomic: Lancaster, PA, 1995.

Tsuda, T.; Ohshima, K.; Kawakishi, S.; Osawa, T. Antioxidant pigments isolated from the seeds of *Phaseouls vulgaris*. *J. Agric. Food Chem*. **1994**, *42*, 248−251.

Vargas-Arispuro, I.; Sanz, B. I.; Martínez-Téllez; M. A.; Primo-Yúfera, E. Actividad antioxidante de compuestos aislados del residuo no-volátil del aceite esencial de naranja. *Grasas Aceites* **1998**, *49*, 159−164.

Visioli, F.; Romani, A.; Mulinacci, N.; Zarini, S.; Conte, D.; Vincieri, F. F.; Galli, C. Antioxidant and other biological activities of olive mill wastewaters. *J. Agric. Food Chem*. **1999**, *47*, 3397−3401.

von Gadow, A.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. *J. Agric. Food Chem*. **1997a**, *45*, 632−638.

von Gadow, A.; Joubert, E.; Hansmann, C. F. Effect of extraction and additional heating on the antioxidant activity of rooibos tea (*Aspalathus linearis*) extracts. *J. Agric. Food Chem*. **1997b**, *45*, 1370−1374.

von Gadow, A.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chem*. **1997c**, *60*, 73−77.

Watanabe, M.; Ohshita, Y.; Tsushida, T. Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Möench) hulls. *J. Agric. Food Chem*. **1997**, *45*, 1039−1044.

Wu, K.; Zhang, W.; Addis, P. B.; Epley, R. J.; Salih, A. M.; Lehrfeld, J. Antioxidant properties of wild rice. *J. Agric. Food Chem*. **1994**, *42*, 34−37.

Wulff, H. Natural antioxidant composition. Int. Patent WO9739632, 1997.

Yamaguchi, F.; Yoshimura, Y.; Nakazawa, H.; Ariga, T. Free-radical scavenging activity of grape seed extract and antioxidants by electron spin resonance spectrometry in an H_2O_2/NaOH/DMSO system. *J. Agric. Food Chem*. **1999**, *47*, 2544−2548.

Yen, G.-C.; Duh, P. D.; Tsai, C. L. Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem*. **1993**, *41*, 67−70.

Yen, G.-C.; Duh, P. D. Antioxidant activity of methanolic extracts of peanut hulls from various cultivars. *J. Am. Oil Chem. Soc*. **1995**, *72*, 1065−1067.

Yen, G.-C.; Chen, H.-Y.; Peng, H. H. Antioxidant and pro-oxidant effects of various tea extracts. *J. Agric. Food Chem*. **1997**, *45*, 30−34.

Yi, O. S.; Han, D.; Shin, H. K. Synergistic antioxidative affects of tocopherol and ascorbic acid in fish oil/lecithin/water system. *J. Am. Oil. Chem. Soc.* **1991**, *68*, 881−883.

Xing, Y.; White, P. Identification and function of antioxidants from oat groats and hulls. *J. Am Oil Chem. Soc*. **1997**, *74*, 303−307.

Zandi, P.; Gordon, M. H. Antioxidant activity of extracts from old tea leaves. *Food Chem*. **1999**, *64*, 285−288.

Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. **1999**, *64*, 555−559.

Received for review January 6, 2000. Revised manuscript received June 16, 2000. Accepted June 16, 2000. This work was funded by the INCO-DC Program of European Commission (ERBIC 18 CT 970206) and cofunded by Xunta Galicia-University Vigo.
JF000048W