CHEMICAL ENGINEERING TRANSACTIONS Volume 17, 2009
Editor Sauro Pierucci
 ISBN 978-88-95608-01-3 ISSN 1974-9791
DOI: 10.3303/CET0917143

Chestnut Burs as a Source of Natural Antioxidants

G. Vázquez, J. González-Álvarez*, M. S. Freire, A. Fernández-Agulló,
J. Santos and G. Antorrena
Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela
Rúa Lope Gómez de Marzoa s/n, 15782 Santiago de Compostela, Spain

The potential of chestnut bur as a source of natural antioxidants has been studied. Extracts in solvents of varying polarity (acetone, ethanol, methanol and water) were obtained and compared with respect to extraction yield, total phenols content, antioxidant activity, molecular weight distribution and composition. The extraction with water led to the highest extraction yield (21.2%) and extract total phenols content (37.6 g GAE/100g), FRAP antioxidant activity (2120 nmol AAE/mg extract), DPPH and ABTS radical scavenging activity (3.68 and 3.59 mmol TRE/g extract, respectively) together with the lowest EC_{50} values for the DPPH (0.044 mg/mL) and ABTS (0.337 mg/mL) essays. The extraction yield, the extract total phenols content and the antioxidant activity (FRAP, DPPH and ABTS) decreased when the polarity of the solvent decreased (water >methanol>ethanol>acetone) and the EC_{50} values increased in the same order. Chestnut bur extracts have been characterized as hydrolysable gallotannins as revealed its MALDI-TOF spectrum showing a major peak series exhibiting a mass increment of 152 Da corresponding to the repeat unit of the galloyl group. In addition to gallic acid esters of glucose, ellagic acid, quercetin, quercetin-3β-D-glucoside and vescalagin/castalagin were found in chestnut bur extracts.

1. Introduction

The need to replace synthetic antioxidants used in the pharmaceutical, cosmetic and food industries whose security has been questioned has promoted the research on new sources of antioxidant compounds. The investigations have been directed towards different vegetable materials but focussed mainly on those undervalued forest or industrial by-products that could be an inexpensive source of natural antioxidant compounds. Among these, different nuts and their by-products have been analysed in literature: *Gevuilla avellana* hull (Moure et al., 2000), almond hull (Pinelo et al., 2004), hazelnut by-products (Shahidi et al., 2007; Contini et al., 2008), pecan by-products (Villareal-Lozoya, 2007), chestnut fruit and by-products (Barreira et al., 2008; Vázquez et al., 2008a, b). Among the isolated compounds, high and low molecular weight polyphenols represent the main class of natural antioxidants.
The Galician (NW Spain) production of chestnut (*Castanea sativa*) averages 22,000 t/year and approximately 7,000 t are processed by the food industry to produce several derivatives such as marron glaçé, chestnut pureé, etc. As a result of chestnut harvesting

* Corresponding author: e-mail: julia.gonzalez@usc.es

Please cite this article as: Vazquez G., Gonzalez-Alvarez J., Freire M.S., Fernandez-Agullo A., Santos J. and Antorrena G., (2009), Chestnut burs as a source of natural antioxidants, Chemical Engineering Transactions, 17, 855-860 DOI: 10.3303/CET0917143

and shelling two waste products are left, the bur and the shell, the latter currently used as fuel. The use of chestnut shell as a source of antioxidant compounds has been previously studied by the authors (Vázquez et al., 2008a, b). The aim of this work is to evaluate the potential of chestnut bur as a source of natural antioxidants. Solvents of varying polarity were used: acetone, ethanol, methanol and water and the extracts compared with respect to extraction yield, total phenols content and antioxidant activity using different essays. In addition, molecular weight distribution was analysed by gel permeation chromatography (GPC), the extracts were characterized by MALDI-TOF spectrometry and the major phenolic compounds identified by RP-HPLC-ESI-TOF.

2. Materials And Methods

2.1 Extraction And Concentration

Chestnut bur was collected in a chestnut plantation in Santiago de Compostela (Galicia, NW Spain). It was air-dried till equilibrium humidity, ground and the fraction of particle size between 0.1 and 2 mm was selected. Extractions with acetone, ethanol and methanol were done in a Soxhlet apparatus for 15 h. The solvent was evaporated in a Büchi R-210 rotavapor. The extraction with water was performed in a 2-L Pyrex glass reactor with mechanical stirring and temperature control at a solid/liquid ratio of 1/10 (w/w), temperature 90ºC and time 1 h. The extract was concentrated by spray-drying. The extraction yield was calculated as the percentage weight loss of the starting material.

2.2 Total Phenols Content

Total phenols content was determined by the Folin-Ciocalteu method: to 0.5 mL of extract aqueous solution, 2.5 mL of Folin-Ciocalteu reactive, diluted with water (1:10, v/v), and 2 mL of a 75 g/L Na_2CO_3 aqueous solution were added. The mixture was kept 5 min at 50ºC and, after cooling, the absorbance at 760 nm was measured. The phenols content was expressed as g gallic acid equivalent (GAE)/100 g extract (on dried basis).

2.3 Antioxidant Activity

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was done as follows: 0.1 mL of extract aqueous solution were transferred to a test tube and 3.0 mL of freshly prepared FRAP reagent (25 mL acetate buffer, 300 mmol/L, pH=3.6; 2.5 mL 10 mmol TPTZ in 40 mmol/L HCl; 2.5 mL 20 mmol/L $FeCl_3 .6H_2O$) were added. The absorbance was recorded after 5 min at 593 nm. The relative activities of samples were expressed as nmol ascorbic acid equivalent (AAE) per mg extract (on dried basis).

DPPH Radical-Scavenging Activity

Aqueous solutions of chestnut bur extracts (8-240 μg/mL) were prepared. The extract solution (0.3 mL) was mixed with 2.7 mL of a freshly prepared DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (6.10^{-5} M in 80% methanol). The mixture was shaken vigorously and left to stand for 20 min in the dark at room temperature. Then the absorbance was read at 517 nm. The radical-scavenging activity (RSA) was determined as $\%RSA=100\ (A_0-A_s)/A_0$, where A_s is the absorbance of the extract solution and A_0 is the absorbance of a control solution prepared without extracts. The extract concentration

that causes 50% loss of the DPPH activity (EC_{50}) was obtained from a graph of %RSA against extract concentration. The Trolox equivalent of the extracts (TRE) was calculated and expressed as mmol Trolox equivalent (TRE) per g extract (on dried basis).

ABTS Radical-Scavenging Activity

ABTS (2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) was produced by reacting an ABTS solution (7 mM) with potassium persulfate (2.45 mM) for 16 h in the dark at room temperature. The $ABTS^{\bullet+}$ solution was diluted with water to an absorbance of 0.70 at 734 nm. Aqueous solutions of chestnut bur extracts (20-1000 µg/mL) were prepared. The extract solution (25 µL) was mixed with the $ABTS^{\bullet+}$ solution (2.5 mL) and after 6 min in the dark at room temperature the absorbance was read at 734 nm. The RSA of the extract solutions, the EC_{50} values and the extracts TRE were calculated as indicated in the DPPH method.

2.4 Molecular Weight Distribution And Average-Molecular-Weights By GPC

Acetylated extracts (Vázquez et al., 2008a) were dissolved in THF (2-5 mg/mL) and analysed by GPC with an Agilent Technologies 1100 chromatograph equipped with a diode array detector. The column used was a HP-PL gel 5µm Mixed-D protected with a PL gel 5µm guard column. THF was used as eluent and the conditions used were: flow rate, 1 mL/min; column temperature, 30ºC; injection volume, 20 µL; detection at 270 nm with a bandwidth of 15 nm. The calibration curve was obtained with polystyrene standards.

2.5 MALDI-TOF spectrometry

The spectra were recorded on a Bruker Autoflex MALDI-TOF instrument equipped with a N_2 laser (337 nm). The measurements were carried out using positive polarity and reflectron mode (1-4 kDa). The samples were disolved in trifluoroacetic acid and 2,5-dihydroxy benzoic acid was used as matrix. For the enhancement of ion formation INa was added to the matrix.

2.6 RP-HPLC-ESI-TOF

The samples were evaluated using an Agilent Technologies 1100 HPLC and a Bruker Microtof ESI-TOF instrument. Tannins were separated using a Zorbax Eclipse XDB-C18 5 µm (4.6x150 mm) column and a binary gradient of 2% acetic acid for mobile phase A and 0.5% acetic acid in water/acetonitrile (1:1, v/v) for mobile phase B at a flow rate of 1 mL/min and a postcolumn split of 1/0.2 mL/min. The linear gradient was from 10 to 55% B from 0 to 50 min, from 55 to 100% B from 50 to 60 min and from 100 to 10% B from 60 to 65 min. The mass spectrometry analysis was performed in negative ion mode.

2.7 Statistical Analysis

All the essays were carried out in triplicate and the results expressed as mean value and standard deviation. The regression analysis between variables was performed with STATGRAPHICS 5.1. With the same software, one-way ANOVA was used to analyze the existence of significant differences between the means by means of the F-test ($p<0.05$). The multiple range test applied a multiple comparison analysis to the data to determine which means are significantly different using the Tukey's HSD test.

3. Results And Discussion

Table 1 shows for the different solvents essayed the results obtained for the extraction yield together with some extract characteristics: total phenols content, antioxidant activity evaluated using various methods and number (M_n) and weight (M_w) average-molecular-weights. The DPPH and ABTS EC_{50} values are presented in Fig. 1a.

A significant content of total phenolics and a good antioxidant capacity were found for all bur extracts, except for the acetone ones. The extraction with water led to the highest extraction yield, extract total phenols content, FRAP antioxidant activity, DPPH and ABTS radical scavenging activities and to the lowest DPPH and ABTS EC_{50} values. The extraction yield, the extract total phenols content and the antioxidant activity decreased when the polarity of the solvent decreased (water > methanol > ethanol > acetone) and the EC_{50} values increased in the same order. Extraction yield and ABTS RSA demonstrated dependence on the solvent used for the extraction. However, ethanol and methanol extracts did not showed significant differences for total phenols contents, and methanol and water extracts for FRAP and DPPH antioxidant activities.

The extraction yields for chestnut bur were significantly higher than those obtained for the shell with the same solvents (from 0.69% for acetone extract to 12.20% for water extract; Vázquez et al., 2008a). With respect to the total phenols yields referred to the raw material, the results obtained for chestnut bur extracts (0.298-7.995 g GAE/100 g material) were of the same order of those encountered for chestnut shell extracts (0.596-6.808 g GAE/100 g; Vázquez et al. 2008 a), slightly lower than those obtained for the extracts of different chestnut by-products (2.15-10.26 g GAE/100 g; Barreira et al., 2008) and for hazelnut skin extracts (2.03-15.2 g GAE/100 g; Contini et al., 2008) but significantly higher than those reported for *Gevuina avellana* hull (0.00103-0.00423 g GAE/100g; Moure et al, 2000) almond hull (0.0077-0.0721 g GAE/100g; Pinelo et al., 2004) and hazelnut shell extracts (0.15-0.21 g GAE/100 g; Contini et al., 2008).

The FRAP antioxidant activities of ethanol and methanol chestnut bur extracts were considerably higher than those of chestnut shell extracts, however, water chestnut extract showed the highest value (Vázquez et al., 2008a). The ABTS RSA of bur extracts determined by the ABTS method were significantly higher than those obtained

Table 1 Extraction yield and characteristics of chestnut bur extracts.

Solvent	Yield	Total phenols	Antioxidant activity			M_n	M_w
			FRAP	ABTS	DPPH		
	(% bur)	(g GAE/ 100 g extract)	(nmol AAE /mg extract)	(mmolTRE /g extract)	(mmolTRE /g extract)	(Da)	(Da)
Acetone	2.43 ^{A} (0.25)	12.27 ^{A} (0.79)	379 ^{A} (11)	0.70 ^{A} (0.07)	1.54 ^{A} (0.27)	1052 ^{A} (37)	1939 ^{A} (65)
Ethanol	6.54 ^{B} (0.38)	27.85 ^{B} (0.93)	1047 ^{B} (32)	1.53 ^{B} (0.10)	2.00 ^{B} (0.24)	1050 ^{A} (1)	1734 ^{B} (4)
Methanol	11.05 ^{C} (0.15)	28.91 ^{B} (1.21)	2060 ^{C} (20)	2.11 ^{C} (0.09)	3.26 ^{C} (0.29)	1232 ^{B} (8)	2062 ^{C} (17)
Water	21.24 ^{D} (0.72)	37.64 ^{C} (1.74)	2120 ^{C} (73)	3.59 ^{D} (0.13)	3.68 ^{C} (0.22)	1388 ^{C} (5)	2122 ^{C} (6)

Mean (Standard deviation); In each column different letters mean significant differences ($p<0.05$)

for hazelnut by-products extracts (0.117-0.148 mmol TRE/g extract; Contini et al. 2008). In relation to the EC_{50} values, they were considerably lower for the DPPH essay in comparison with the ABTS one, being the former of the order of those obtained for chestnut by-products (0.075-0.170 mg/mL; Barreira et al., 2008).

As illustrated in Fig. 1b, a high correlation was found between DPPH and ABTS EC_{50} values and total phenols content (r^2 = 0.9043, p=0.0491 and r^2 = 0.9697, p=0.0153, respectively). The relationship between antioxidant capacity and total phenols content has been also reported for extracts of other plant materials (Barreira et al., 2008; Vázquez et al., 2008a, b). On the other hand, the data obtained using the FRAP and the DPPH essays were well correlated (R^2=0.9614, p=0.0195) whereas the ABTS and DPPH values (R^2=0.8679, p=0.0684) and the FRAP and ABTS values (R^2=0.7772, p=0.1182) were less well correlated.

The extracts molecular weight distribution is shown in Fig 2. Acetone, ethanol and methanol extracts showed two distinct peaks and some resolution of higher molecular weight species, whereas for water extracts the low molecular peak is hardly resolved. As a result, the highest mean (M_n) and weight (M_w) average-molecular-weights were obtained for water extracts and both diminished, in general, when the polarity of the solvent was reduced. Additionally, both average molecular weights were significantly lower than those obtained for chestnut shell extracts (Vázquez et al., 2008a), which could be related with the different chemical nature of the constituent tannin extracts since chestnut shell extracts are of condensed character. However, water chestnut bur extracts have been characterized as gallotannins as revealed its MALDI-TOF spectrum showing a major peak series exhibiting a mass increment of 152 Da corresponding to the repeat unit of the galloyl group.

Tannins were analysed by RP-HPLC-ESI-TOF, which revealed that chestnut bur extracts mainly included gallic acid esters of glucose and ellagic acid, small proportions of quercetin, quercetin-3β-D-glucoside and vescalagin/castalagin (water and ethanol extracts) together with some not identified compounds.

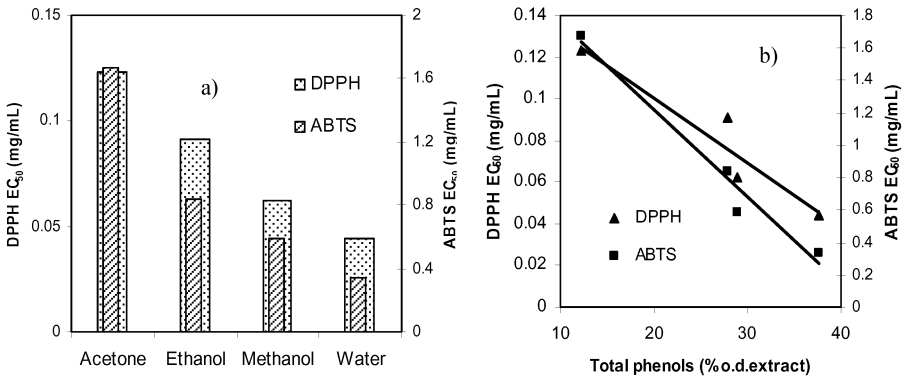

Fig. 1. DPPH and ABTS EC_{50} values for chestnut bur extracts in different solvents a) and correlation of DPPH and ABTS EC_{50} values and total phenols content b).

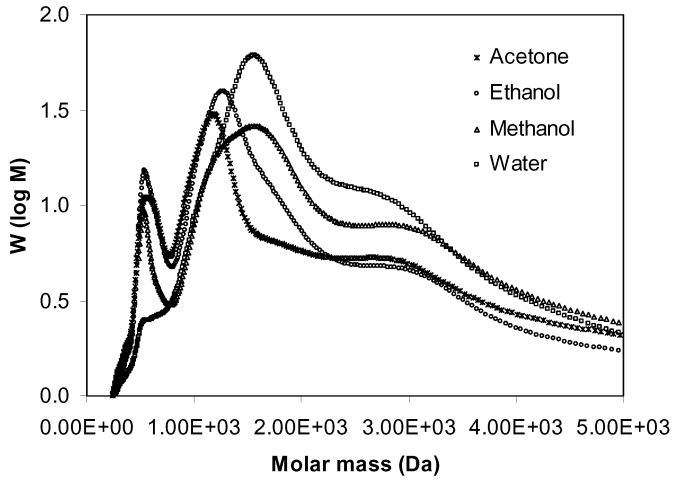

Fig. 2. Molecular weight distribution obtained by GPC for acetylated chestnut bur extracts of various solvents.

References

Barreira, J.C.M., Ferreira, I.C.F.R., Oliveira, M.B.P.P., Pereira, J.A., 2008, Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit, Food Chem. 107, 1106-1113.

Contini, M., Baccelloni, S., Massantini, R., Anelli, G., 2008, Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature, Food Chem. 110, 659-669.

Moure, A., Franco, D., Sineiro, J., Dominguez, H., Núñez, M.J., Lema, J.M., 2000, Evaluation of Extracts from *Gevuina avellana* Hulls as Antioxidants, J. Agric. Food Chem. 48, 3890-3897.

Pinelo, M., Rubilar, M., Sineiro, J., Núñez, M.J., 2004, Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*), Food Chem. 85, 267-273.

Shahidi, F., Alasalvar, C., Liyana-Pathirana, C.M., 2007, Antioxidant phytochemicals in hazelnut kernel (*Corylus avellana* L.) and hazelnut byproducts, J. Agric. Food. Chem. 55, 1212-1220.

Vázquez, G., Fontenla, E., Santos, J., Freire, M.S., González-Alvarez, J., Antorrena, G., 2008a, Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts, Ind. Crops Prod. 28, 279-285..

Vázquez, G., J. González-Alvarez, J. Santos, M.S. Freire, G. Antorrena, 2008b, Evaluation of potential applications for chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts, Ind. Crops Prod., in press, doi:10.1016/j.indcrop.2008.07.004.

Villarreal-Lozoya, J.E., Lombardini, L., Cisneros-Zevallos, L., 2007, Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars, Food Chem. 102, 1241-1249.