Industrial Crops and Products 36 (2012) 172–176

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Valorization of chestnut husks by non-isothermal hydrolysis

Noelia González López, Andrés Moure*, Herminia Domínguez, Juan C. Parajó

Departamento de Enxeñería Química, Facultade de Ciencias de Ourense, Universidade de Vigo, Edificio Politécnico, As Lagoas, 32004 Ourense, Spain

ARTICLE INFO

Article history:
Received 23 June 2011
Received in revised form 31 August 2011
Accepted 6 September 2011
Available online 20 October 2011

Keywords:
Chestnut husks
Autohydrolysis
Alkaline hydrolysis
Ethyl acetate
Antioxidants
Membranes

ABSTRACT

Chestnut is a valuable fruit with traditional food applications due to its nutritional value. The shell accounts for around 10% of the fruit weight and have no commercial applications, so they are destined for fuel. The development of alternative applications for the shells could contribute to valorization of the major constituents and to an integral utilization of renewable resources of low cost and residual origin according to a sustainable development philosophy.

Husks come from two different peeling treatments, a dry dehusking process and a wet dehusking process. Husks from the dry process contained more lipophilic compounds as those hulls coming from the wet dehusking process.

Treatments of chestnut husks were performed to release tannins and hemicelluloses components. High temperature extraction (HTE) was the selected technology, and aqueous and alkaline media were the solvents chosen. Treatments allowed the solubilisation of 37.7% of the chestnut husks, with maximum values of 55.5% of phenolic compounds solubilisation at treatment temperature of 180 °C. The higher antioxidant activity had a maximum value of 1.87 g Trolox/gram of extract at 160 °C.

The extract produced under the optimal processing alkaline conditions was processed by membrane technology and the phenolic concentration in the final product was about 15% higher than the initial phenolic concentration in the hydrolyzates. This product showed an antioxidant activity comparable to that of synthetic antioxidants.

1. Introduction

Chestnut (*Castanea sativa*) is a valuable fruit with traditional food applications due to its nutritional value. The shell accounts for around 10% of the fruit weight and in the industrial manufacture of marron glacé and chestnut purée the chestnut shell is removed during the peeling stage. At present, shells have no commercial applications and are destined for fuel. The development of alternative applications for the shells could contribute to valorization of the major constituents and to an integral utilization of renewable resources of low cost and residual origin according to a sustainable development philosophy.

The extraction of phenolic compounds from chestnut hulls using organic solvents (Vázquez et al., 2008) and the extraction with alkaline aqueous solutions (sodium sulfite, sodium carbonate and sodium hydroxide) were reported (Vázquez et al., 2009, 2010). These authors proposed the application of the extracted phenolics as phenol substitutes in the formulation of adhesives for wood derivatives, as chrome substitutes in leather tanning and as antioxidants. During the alkaline extraction of phenolic compounds the temperature selected was 70–90 °C, in the range of those used in industrial scale for the extraction of tannins for wood adhesives and leather tanners. However, the need for chemical free processes and the successful utilization of high pressure water extraction processes for the extraction of food antioxidants (Garrote et al., 2003, 2004, 2008; Conde et al., 2008, 2011) could suggest the suitability of trying higher temperatures in the absence of chemicals; an alternative not previously reported for this material.

Autohydrolyis is an environmentally friendly technology for biomass fractionation. The catalytic species responsible for the hydrolytic degradation of hemicelluloses are the hydronium ions from water autoionisation and the organic acids generated in the process. The resulting liquors contain a mixture of sugar oligomers, monosaccharides, sugar-decomposition products and acetic acid. The solid phase from treatments is enriched in cellulose, which is hardly altered during autohydrolysis, enabling its further utilization for a variety of purposes. The autohydrolysis processing of chestnut shells has not been studied and it could be advantageous not only due to operational and environmental reasons. Despite the higher phenolics extraction yield at higher pH, the extraction selectivity was enhanced at lower pH, allowing higher purity, Stiasny number and antioxidant activity of the tannin extract (Vázquez et al., 2009).

The present work is aimed at assessing the feasibility of a non isothermal treatment to solubilise the hemicelluloses and tannin fractions of chestnut husks and to recover fractions with

* Corresponding author. Tel.: +34 988368892; fax: +34 988387001.
E-mail address: amoure@uvigo.es (A. Moure).

0926-6690/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2011.09.002

antioxidant activity in an attempt to valorize industrial residues as raw materials for high-value products.

2. Materials and methods

2.1. Raw material

Chestnut husks were collected from a chestnut processing plant (*Marrón Glacé*, San Cibrao das Viñas, Ourense, Spain). Husks and their cuticles were manually separated and shells were ground to about 1 mm with an arms mill, homogenized in a single lot and stored in a dark, dry place until use.

2.2. Hydrolytic processing

2.2.1. Hydrolytic processes and solvent extraction of liquors

Chestnut husks were contacted with water and/or alkaline solutions in a batch reactor with temperature control using a liquid to solid ratio of 8 kg:kg (oven-dry basis) and heated to the desired temperature (in the range 185–260 °C) following the standard temperature profile of the reactor (Garrote et al., 2003). After cooling, the liquid phase was separated by vacuum filtration and the hydrolyzate was assayed for total soluble solids (extraction yield), phenolic compounds and antioxidant activity.

2.3. Experimental membrane set-up

Commercial membrane Nanomax was selected on the basis of previous surveys with other materials in order to concentrate phenolic compounds present in few hydrolyzates (Díaz-Reinoso et al., 2009). The equipment used consists of a 10 L feed tank (in which temperature was controlled by flushing tap water through a refrigeration coil), a variable speed Hydracell pump, two pressure gauges at the membrane inlet and outlet to measure the transmembrane pressure (TMP), a needle valve located after the membrane to achieve the desired TMP, and a flowmeter to measure the recycle flow. Operation was carried out at room temperature (20 °C) and at the TMP was selected to avoid fouling membrane.

The retentate streams from the Nanomax membrane were subjected to ultrafiltration with variable volume diafiltration in an Amicon membrane with a 1 kDa cut-off in order to remove sugar derivates and alkaline media. In this work, the concentration ratio in diafiltration operation was 3 and the process was repeated three times.

2.4. Analytical methodology

2.4.1. Raw material and solid residue composition

The composition of the raw material and spent solids from hydrothermal treatments was determined by quantitative acid hydrolysis with 72% sulfuric acid following standard methods (Browning, 1967). Liquors were assayed for sugars, acetic acid, furfural and hydroxymethylfurfural by HPLC (Conde et al., 2008).

2.4.2. HPLC analysis of sugars

The liquors of hydrothermal processes and the liquors hydrolyzated from acid post-hydrolysis were analyzed by HPLC using a Biorad Aminex HPX-87H column eluted with 0.003 M H_2SO_4

2.4.3. Colorimetric determination of total phenolics

Total phenols were determined spectrophotometrically at 765 nm using with the Folin–Ciocalteau reagent. Absorbances were compared with a standard curve and expressed as gallic acid equivalents (GAE).

2.4.4. Antioxidant activity

The antioxidant activity of extracts was measured by *Trolox equivalent antioxidant capacity* (*TEAC*). This assay is based on the scavenging of ABTS radical [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)]. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS stock solution with potassium persulfate at 2.45 mM final concentration, maintained in the dark for 12–16 h and then diluted with phosphate buffer saline (PBS) (pH 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. After the addition of 1.0 mL of diluted $ABTS^{\bullet+}$ solution to 10 mL of antioxidant compounds or Trolox standards in PBS, the absorbance readings were taken up to 6 min. The radical scavenging capacity was expressed as Trolox equivalents using a standard curve.

Ferric reducing antioxidant power (*FRAP*). The reagent was prepared by mixing 25 mL of 300 mmol/L acetate buffer (pH 3.6) and 2.5 mL of a 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mmol/L HCl and 20 mmol/L $FeCl_3{\cdot}6H_2O$ in distilled water. Samples (100 mL) were mixed with the reagent (3 mL), and the absorbance was monitored at 593 nm; $FeSO_4$ aqueous solutions were used for calibration.

Reducing power. One milliliter of extract (dissolved in methanol) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1.0% potassium ferricyanide, and the mixture was incubated at 50 °C for 30 min. After adding 10% trichloroacetic acid (2.5 mL), the mixture was centrifuged and the supernatant (2.5 mL) was mixed with water (2.5 mL) and 0.1% ferric chloride (0.5 mL). Absorbance was read at 700 nm and the results are expressed as ascorbic acid equivalents.

β-Carotene bleaching method. The spectrophotometric method of Miller (1971) was used. The antioxidant activity antioxidant activity coefficient (AAC), which measures the relative oxidation in the presence of extracts respect to the oxidation in their absence was calculated as:

$$\frac{\text{absorbance of extract}_{120\ \text{min}} - \text{absorbance of control}_{120\ \text{min}}}{\text{absorbance of control}_{0\ \text{min}} - \text{absorbance of control}_{120\ \text{min}}} \times 1000$$

The antioxidant activities of the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were measured under the same conditions for comparison data. All tests and analyzes were run in triplicate, and the average values are presented.

ORAC-FL assay. The original method of Ou et al. (2001) was modified as follows: the reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 mL. The mixture of antioxidant (20 mL) and fluorescein (120 mL; 70 nM final concentration) was preincubated for 15 min at 37 °C. AAPH (2,2′-azobis (2-methylpropionamidine) dihydrochloride) solution (60 mL; 12 mM, final concentration) was added rapidly. Fluorescence was recorded during 120 min (excitation wavelength 485 nm, emission wavelength 520 nm) in a fluorescence spectrophotometer. Results were calculated from the differences in areas under the decay curve for the blank and the sample, and are expressed as Trolox equivalents using a standard curve (2–40 mM Trolox). Assays were performed in triplicate.

2.4.5. Stiasny number

The content of formaldehyde condensable polyphenols, expressed as the Stiasny number, was determined according to Paridah et al. (2002). Liquors from alkaline processing (50 mL) were reacted with an aqueous formaldehyde solution (37%; 10 mL) and a 10 M hydrochloric acid solution (5 mL) in a 150 mL glass flask under reflux for 30 min and filtered through a fritted glass crucible (porosity 2). The precipitate is washed with warm distilled water and oven-dried at 105 °C to constant weight.