

Tannins Extraction from Walnuts Residues

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Tannins are natural water-soluble products, characterized by a phenolic structure and by the ability to bind and precipitate proteins. They are widely found in natural products, and their "historical" utilization was to convert animal hides into leather. Nowadays tannins are extensively used in food and beverage industry and in pharmaceutical and nutraceutical industry for their positive effects on human health.

In conventional processes tannins are extracted from vegetable material by using water as solvent in a temperature range from 40 to 90 °C; other polyphenols are always extracted and are classified as non tannins.

Scope of this work is to characterize residue (in particular the woody septum) of walnut core in terms of total extractable tannins. To this aim chemical analytic methods, reported in the literature, for the quantitative determination of these compounds in aqueous solution of unknown composition have been assessed.

Total extractable tannins in woody septum have been determined by means of an experimental procedure developed in previous works focused on tannins extraction from chestnut wood. The evaluation of extractable tannins is very important to determine the feasibility of a process to extract tannins from this type of residue. Furthermore experimental data on equilibrium distribution of tannins between solid (woody septum) and liquid (water) at temperature of 80°C have been collected. The obtained equilibrium data have been correlated by means of a linear adsorption isotherm.

1. Introduction

In recent years, tannins production has become a very important issue because of their increasing commercial interest in the field of pharmaceutical, food and nutraceutical industries. In particular, tannins can contribute to the therapeutic effects of some herbal medicines; beverages rich in tannins have positive cardiovascular effects and, like some other smaller phenolic compounds, tannins may serve as dietary antioxidants. Finally, as it is well known, tannins are used for converting animal hides to leather, owing to their ability to interact with and precipitate proteins (He et al. 2007, Mateus et al. 2004). Extraction process of tannins from natural matrix is nowadays performed by empirical methods, and industrially no optimization of extraction process has been carried out. In order to maximize the recovery of tannins from the solid matrix, the influence of feed pre-treatment (crushing and moisture removal), temperature and extraction time, chemical characteristics of the solvent and solvent to solid ratio should be extensively

studied (Guerrero et al., 2008). In particular, the optimization of the contact time and extraction temperature should be a powerful tool to reduce the energy consumption of the process.

Water is the most extensively studied solvent for the tannins extraction, but also organic solvents such as methanol, ethanol, ethylacetate or acetone and aqueous solutions of the same organic compounds are employed. Furthermore the possibility of utilizing carbon dioxide as non-toxic, environmentally safe, cheap solvent has been investigated by Murga et al. (2001) and Luengthanaphol et al. (2004).

In this work, some experimental data on tannins extraction from walnut septum, utilizing water as solvent at a fixed temperature, are reported. The choice of water as solvent was made on the basis of literature data: in fact it was verified that the extraction efficiencies of organic solvents are comparable with that of water, which is less expensive and polluting (Gironi and Piemonte in press, Gironi et al. in press).

The results obtained can be considered an helpful tool for the optimization of the existing extraction processes of tannins from solid matrix and for the design of production plants.

2. Materials and Methods

Experimental runs on tannins extraction were performed on samples of walnut septum derived from walnut residues kindly purchased from Cofrus Industry (Italy). The walnut septum were crushed, then placed in a vacuum drying oven for a period of approximately 48 hours at a temperature of 90 °C in order to remove the moisture initially present. It was measured an initial moisture content equal to 0.098 g-H₂O / g-dry-wood.

The concentration of tannins in aqueous solutions was determined by means of protein (bovine serum albumin, BSA provided by Sigma-Aldrich) precipitation assay. This method requires an aqueous solution containing 5% v / v triethanolamine (TEA) and 1% v / v of sodium dodecyl sulphate (SDS) provided by Sigma-Aldrich. All the chemicals used were reagent grade.

The details about the analytical procedure followed for determining the concentration of tannins in solution are reported in literature (Schofield and Mbugua, 2001, Rautio et al. 2007). All the liquid samples collected were analysed by means of a Perkin-Elmer Lambda 25 spectrophotometer. Calibration was obtained by measuring the absorbance of solutions of known composition at the wavelength of 522 nm. An extinction coefficient $\epsilon=0.5801$ was obtained.

The experimental setup used for the tests consists of a glass column (H=0.5 m, $\Phi=0.049$ m) equipped with a thermostatic jacket, a peristaltic pump for the flow recirculation, a drum for the product accumulation and a thermostat to maintain the temperature at a fixed value (see figure 1). The experimental setup, working in closed loop, was used to carry out both the equilibrium and characterization tests.

The equilibrium tests were made at 80 °C by loading dried and milled walnut septum inside the column and by adding the solvent (deionised water) inside the accumulation

drum. After the loading phase, the pump was turned on thus enduring the solvent circulation in the column and the contact between the solid and liquid phase.

By means of some preliminary tests it was shown that a contact time equal to about 24 h was necessary to reach equilibrium conditions.

At the end of each experimental run, the tannins concentration in the liquid phase was determined by the analytical procedure above reported.

3. Results

The experimental work was firstly devoted to the evaluation of tannins concentration in walnut septum. The test, performed at 80 °C, required a series of extraction runs on the same wood septum, performed in the experimental apparatus. In each run, when equilibrium conditions were reached, the liquid solution was removed, analysed and replaced with fresh solvent. Naturally, owing to the transfer of tannins from solid to liquid phase, the concentration of tannins in the liquid phase decreases as the number of extractions increases. The last extraction always showed a concentration of tannins in liquid phase around the detectable limit of the analytical method used to determine tannins concentration.

By means of a material balance extended to all the extraction runs, it was possible to evaluate the mass of extractable tannins. Therefore, the amount of extractable tannins present in the sample, N_{in} , was evaluated by:

$$N_{in} = \sum_{i=1}^n V_i C_{i,eq} \quad (1)$$

where V_i and $c_{i,eq}$ stand for a solvent volume loaded in the circuit and tannins equilibrium concentration in the liquid phase for the extraction run i , respectively.

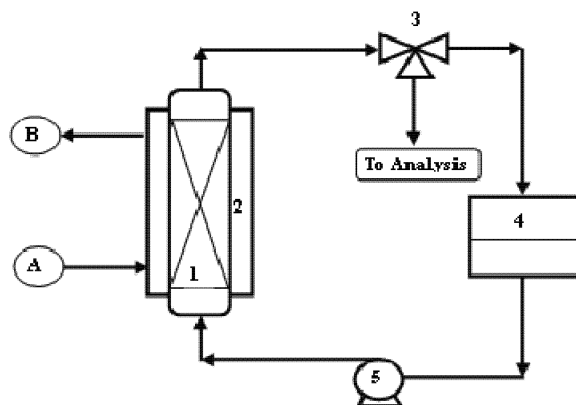


Figure 1. Experimental set-up: (1) Fixed-Bed column, (2) Heating jacket, (3) Three ways valve, (4) Accumulation drum, (5) Circulation pump; (A) from thermostat, (B) to thermostat.

These runs were repeated five times and finally it was obtained a mean value of the extractable content of tannins of about 0.053 grams of tannins on a gram of dry wood (n_{in}).

Afterwards, the experimental work was devoted to the study of equilibrium conditions between solid and liquid phase. Table 1 summarizes the operational conditions used in the experimental runs reported in this paper; different equilibrium conditions were obtained by varying the ratio between the mass of circulating water and the mass of wood septum. Column equilibrium data, such as those reported in the present work, show the disadvantage that only low concentration data can be obtained. Indeed, to obtain data at high solute concentration, low values of the liquid/solid ratio should be used. Unfortunately, solvent volume can not be reduced beyond the liquid hold-up of the experimental apparatus.

Once equilibrium conditions were reached, the concentration of tannins in aqueous solution, C_{eq} , was determined by the analytical method described above, while for the solid phase the equilibrium concentration was calculated from the following material balance equation:

$$M(n_{in} - n_{eq}) = Vc_{eq} \quad (2)$$

where M stands for the total mass of dry walnut septum loaded inside the circuit and n_{eq} is the tannins equilibrium concentrations in the solid phase.

Equilibrium data are summarized in Table 1, while Figure 2 shows a plot of adsorbed tannins amount vs tannins concentration in the liquid phase.

4. Discussion

From a theoretical point of view, the equilibrium data can be correlated by different adsorption isotherms. Since the experimental runs were carried out only for low concentration values of tannins in the liquid phase, in this work a linear isotherm was chosen. Therefore, tannins equilibrium data were correlated by the following linear isotherm:

$$n_{eq} = m \cdot c_{eq} \quad (3)$$

Coupling eq. (2) with eq. (3) we obtain

$$c_{eq} = \frac{M/V \cdot n_{in}}{1 + m \cdot M/V} \quad (4)$$

that is an useful expression to correlate the experimental data when the tannins equilibrium concentrations in the solid phase is very low and therefore highly affected by errors.

The isotherm parameter, m , was evaluated from the fitting of the experimental data by the least square method. The parameter value obtained along with its asymptotic standard error ($R^2=0.95$) is reported in Figure 2, where fitted curve and experimental data are compared. The figure shows that the linear isotherm gives a quite satisfactory correlation of the experimental data. The obtained m value is consistent with data reported in a previous work (Gironi et al., in press).

Table 1: Experimental equilibrium data

Sample	Wood Mass (g)	Solvent volume (l)	Tannins	Tannins
			concentration in the liquid phase (g/l)	concentration in the solid phase (g/g)
1	34.1	0.20	7.97	0.0204
2	30.0	0.50	3.64	0.0064
3	20.0	0.15	6.21	0.0205
4	30.0	0.30	5.88	0.0082
5	30.0	0.40	4.17	0.0152

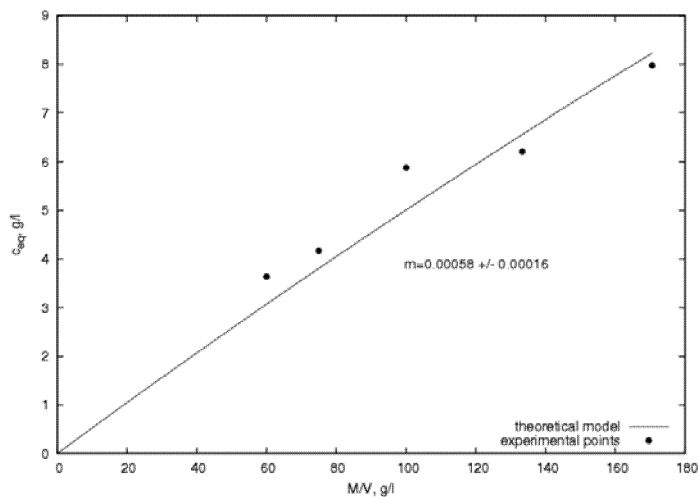


Figure 2: Tannins extraction equilibrium at 80 °C. Line is obtained with the model described in the text (eq.4).

5. Conclusions

This paper deals with an experimental and theoretical study, performed in order to assess the feasibility of an extraction process to recover tannins from walnut septum. Firstly it was proposed a simple procedure to evaluate tannins content in solid matrices: a value of 0.053 g-tannins/g-dried-walnut-septum was obtained. Then, at 80°C, batch equilibrium tests were carried out to obtain information on equilibrium conditions. The hypothesis of an equilibrium condition of tannins between liquid and solid phases was adopted and a linear isotherm was chosen to fit experimental data.

The results reported in this paper can be helpful to the design of industrial processes for the tannins extraction from natural solid matrix.

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