

Optimizing the extraction of phenolic compounds from walnut (*Juglans major* 209 x *Juglans regia*) leaves: a valorization proposal as a source of bioactive compounds

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- 1. Introduction** – *Juglans major* 209 x *Juglans regia* is a walnut hybrid species intended to produce good-quality wood. Although wood is the main product, the use of other fractions that can be considered as wastes, such as the leaves, would contribute to a more profitable production and to a more sustainable plantation management. Walnut leaves have been intensively used in traditional medicine and various studies have demonstrated the bioactivity of the extracts of *Juglans regia* leaves [1, 2]. The aim of this work was to optimize the extraction of bioactive phenolic compounds from the leaves of the abovementioned hybrid species, *Juglans major* 209 x *Juglans regia*, and the phytochemical characterization of the extracts. Looking for green extraction processes [3] extraction was performed by maceration with aqueous ethanol, a generally recognized as safe (GRAS) solvent.
- 2. Experimental** – Walnut (*Juglans major* 209 x *Juglans regia*) leaves were collected in a plantation located in A Coruña (Spain). In a first stage, the influence of the solid-liquid ratio was analysed (1/5-1/10 g/mL) and, once this variable was selected (fixed at 1/10), a Box-Behnken experimental design was applied to analyze the influence of temperature (25, 50 and 75°C), time (30, 75 and 120 min) and aqueous ethanol concentration (10, 50 and 90%) on extraction yield (EY) and extract antioxidant activity (FRAP, DPPH and ABTS methods). The phenolic profile of the extract selected as the optimum was analyzed by UPLC/ESI-Q-TOF-MS.
- 3. Results and Discussion** - Significant models were found for all the dependent variables and the influence of the significant effects was analyzed. FRAP and DPPH were independent of time, while for EY and ABTS the maximum was attained at 120 and 75 min, respectively. With respect to %EtOH, a concentration of 50% led to the maximum for FRAP and ABTS whereas 38% for EY and 44% for DPPH. 75°C was the optimum temperature for all variables except for ABTS (71.25°C). Finally, the operational conditions selected which led a high extract antioxidant capacity and extraction yield were: 75°C, 120 min and 50% aqueous ethanol. The predicted/experimental values for the optimum extract properties were as follows: EY, 30.21/31.92±0.76%, FRAP, 1529/1473±35 nmol AAE/mg extract dw; DPPH, 1.40/ 1.50±0.053 mmol TRE/g extract dw; ABTS, 1.25/1.15±0.011 mmol TRE/g extract dw. The most abundant compound in this extract was the flavanol quercetin-3-β-D glucoside followed by the phenolic acids neochlorogenic and chlorogenic acids.
- 4. Conclusions** - This work proposes a valorization way of walnut leaves as a source of valuable compounds for various industrial applications based on their bioactive properties. Phenolic compounds such as quercetin-3β-D-glucoside, neochlorogenic and chlorogenic acids have been recovered from walnut leaves by maceration in aqueous ethanol solutions under the optimal conditions selected.

5. References

- [1] J.A. Pereira, I. Oliveira, A. Sousa, P. Valentao, P.B. Andrade, I.C.F.R. Ferreira, F. Ferreres, A. Bento, R. S