

EXTRACTION KINETICS OF MARITIME PINE (*PINUS PINASTER*) BARK BY HPE USING CO₂+EtOH

H. C. de Sousa^{1,*}, I. J. Seabra^{1,2}, O. Gomes¹, M. E. M. Braga¹

¹CIEPQPF, Chemical Engineering Department, University of Coimbra, Pólo II, Pinhal de Marrocos, Rua Sílvia Lima, 3030-790 Coimbra, Portugal. Corresponding author email: hsousa@eq.uc.pt. Fax: +351 239 798 795

²ESAC, Politechnic Institute of Coimbra, Bencanta, 3040-316 Coimbra, Portugal

ABSTRACT

Maritime pine (*Pinus pinaster*) bark, an abundant residue rich in high-value phenolic compounds, which has pharmaceutical, food and tanning applications, was subjected to fractionated and non-fractionated high pressure extraction (FHPE and HPE, respectively). Carbon dioxide (CO₂) was the chosen solvent to extract the pine bark volatile fraction and EtOH was added to CO₂ to recover the phenolic fraction. The effect of FHPE solvent overall flow rate was studied on 1st step (CO₂) and 2nd step (CO₂+EtOH) extraction kinetics. Due to the low 1st step yield (0.6-1.0 %, db), HPE was performed with no fractionation, at 30 °C and ~25 MPa. The overall flow rate that yielded the highest global yield ($\sim 7 \times 10^{-5}$ kg/s) was chosen to carry out HPE with different EtOH percentages (30-90 %, v/v). Obtained high pressure extracts were compared with hydrodistillation and Soxhlet extraction results, in terms of global yields, compositions (assessed by thin layer chromatography) and total phenols (quantified by a spectrophotometric assay for the phenolic extracts). Kinetic parameters (M_{CER} , Y_{CER} , and t_{CER}) were obtained using Matlab. The already referred flow rate ($\sim 7 \times 10^{-5}$ Kg/s) yielded the highest global yield for HPE, as observed for both FHPE 1st and 2nd steps. For the 1st FHPE step (CO₂) the highest flow rate originated the lowest M_{CER} , while for the 2nd FHPE step and HPE (using CO₂ + 10 % EtOH) it resulted in the highest one. For the first FHPE step, the extract solubility for the CER period (Y_{CER}) was inversely proportional to the flow rate. Considering the solvent composition effect on M_{CER} and Y_{CER} obtained for HPE, there was an increasing tendency for these kinetic parameters with the increment in the ethanol percentage from 10 to 70 %, probably because of the presence of higher amounts of the solvent liquid phases.

INTRODUCTION

Maritime pine (*Pinus pinaster*) is one of the most important forest species consumed in Portugal by the furniture, wood and pulp and paper industries. Its byproduct (pine bark) is a very promising source of high-value phenolic compounds which can have important applications in food, cosmetic and pharmaceutical industries. Typical phenolic compounds present in pine bark are (+)-catechin, (-)-epicatechin, dihydroquercetin, as well as phenolic acids. Most of these compounds are procyanidin dimers, trimers, oligomers and polymers, i.e. condensed tannins [1]. Pycnogenol® is a commercially available French maritime pine bark extract that was reported to have potent antioxidant activity, and consequently health promoting properties [2].

The choice of the extraction process, solvents and operational conditions is always conditioned by the required extract quality and other particular specifications, like extraction yield and presence of undesired compounds. Conventional solid-liquid extraction with water, alcohols and/or acidified alcohols is usually employed for condensed tannins extractions. However, and because natural products usually contain a wide variety of low and high molecular weight phenolic compounds (and their complexes), natural extracts from these materials will always contain a mixture of different classes of these phenolic substances, depending on the chosen extraction solvent and on the particular employed operational conditions. Usually, additional steps are then required in order to purify/concentrate the desired compounds and to remove the undesired phenolic and other non-phenolic substances. For food and pharmaceutical applications high pressure extraction (HPE) represents an attractive option to conventional solid-liquid extraction and, in some cases, supercritical fluid extraction can also be applied, offering several advantages in terms of selectivity, separation conditions and on the use of environmental friendly technology and solvents.

The main goal of this work is to recover/extract phenolic compounds from pine bark using high pressure extraction methodologies and to compare these procedures results to Soxhlet extraction (SoE) and hydrodistillation (HD) results. Carbon dioxide was the chosen solvent to extract the pine bark volatile fraction, and ethanol (EtOH) was added to CO₂ to obtain the phenolic fractions. The effects of overall solvent flow rate and solvent mixtures compositions were studied on the extraction kinetics results and on the ethanolic extracts compositions.

MATERIALS AND METHODS

Raw material – Comminuted pine bark was provided by a wood processing company from Beira Litoral, Portugal. Particles having a size distribution between 60–18 mesh were separated using sieves under mechanical agitation (Retsch, Germany) and conditioned in plastic bags at approximately -10 °C. Pine bark humidity was determined by the xylol distillation method of Jacobs [3], employing triplicate assays.

Chemicals - Carbon dioxide (99.998 %), ethanol (99.5 %) and distilled water were used for extraction experiments. Analytical grade chemicals and solvents employed for extract analysis were: ethanol, methanol, formic acid, ethyl acetate, hexane, glacial acetic acid, p-anisaldehyde, vanillin, hydrochloric acid, Folin-Ciocalteu's phenol reagent, sodium carbonate, and distilled water. Employed standards were quercetin dehydrate (≥98 %), rutin hydrate (≥95 %), D-(+)-catechin hydrate (98 %), gallic acid (≥98 %) and epicatechin (≥90 %).

Experimental procedure for high pressure extractions – These assays were performed using a supercritical fluid extraction apparatus containing a $\sim 30 \times 10^{-6}$ m³ stainless steel extraction cell [4]. Pressure and temperature conditions were based on the work developed by Seabra et al. [4], taking into account previously obtained pine bark extract yields and compositions. Fractioned and non-fractioned extractions (FHPE and HPE, respectively) were employed, using several operational conditions, as detailed in **Table 1**. Extracts were recovered in a glass flask and a trap, placed in an ice bath. Tubing line was cleaned with EtOH after each extraction or extraction step. Ethanol was then slowly evaporated from extracts using a rotary evaporator and the dried extracts were stored at approximately -18 °C, until further analysis. HPE (CO₂ + EtOH 70 %) assays were performed in duplicate.

Hydrodistillation and Soxhlet extraction – The volatile oil fraction was obtained by HD in a Schilcher apparatus, following the AOAC 962.17 method [5], with a 1:33 (w/v) solid/solvent ratio. The oleoresin (EtOH extract) was obtained in a Soxhlet apparatus, employing a 1:50 (w/v) solid/solvent ratio. Both extraction systems were kept under reflux for 120 min, at the

solvent boiling point (triplicate assays). EtOH was removed from extracts using a rotary evaporator with vacuum control.

Thin Layer Chromatography (TLC) - Analyses of the low polarity compounds present in extracts were performed using silica gel plates (Merck, Germany), using hexane-ethyl acetate (8:2, v/v) as the mobile phase and an anisaldehyde solution as the spray reagent [6].

Table 1: Experimental conditions tested for high pressure extraction methodologies performed with pine bark.

Extraction Methodology	Fractionation	Solvent	T (°C)	P (MPa)	Time (h)	Solvent Flow Rate $\times 10^5$ (kg/s)	
FHPE	Yes	1 st step	CO ₂	50±0.1	20.3±0.3	6.0	6.9±2.3;
		2 nd step					CO ₂ +EtOH (10 %, v/v)
HPE	No	CO ₂ +EtOH (10 %, v/v)	30±0.1	25.3±0.4	6.0	12.1±2.9;	17.3±4.8
HPE	No	CO ₂ +EtOH (30, 50, 70, 90 %, v/v)	30±0.1	25.4±0.3	3.5	7.6±0.3	7.7±2.3;
							14.0±5.0;
							20.8±5.0

Quantification of phenols – Extracts total phenols were quantified according to the Folin-Ciocalteu's method, following the procedure proposed by Singleton and Rossi [7] with modifications [8], and were expressed as gallic acid equivalents.

Calculation Procedures – Each overall high pressure extraction curve was fitted by a curve formed by two lines. The fitting was done by minimizing the least regression error in the least squares sense, using the `fminsearch` function of Matlab (R2007a). The first line was identified with the constant extraction rate period (CER) and the corresponding kinetic parameters were calculated (mass transfer rate for the constant extraction rate period, M_{CER} , mass ratio of solute in solvent phase at measuring-cell outlet, Y_{CER} , and duration of the constant extraction rate period, t_{CER}), according to Rodrigues et al. [9].

RESULTS

Pine bark particles of mean geometric diameter of 0.76×10^{-3} m were used for the extraction experiments, with 3.9 ± 0.17 % (w/w, db) of humidity.

All high pressure extraction curves obtained exhibited the typical constant extraction rate (CER), the falling rate period (FER) and the diffusion controlled rate period (DP), and are represented in **Figure 1** for the non-fractionated assays. The calculated kinetic parameters (M_{CER} , t_{CER} and Y_{CER}) are presented in **Table 2**, as well as global yields obtained during the CER period, R_{CER} .

In general, the solvent flow rate influenced fractionated and non-fractionated extraction kinetics. For the first FHPE step, the highest solvent (CO₂) flow rate resulted in the lowest M_{CER} (constant extraction rate mass) while for the second FHPE step and HPE (performed with CO₂ + 10 % EtOH), it resulted in the highest one.

The CO₂ flow rate had a negative impact on the extract solubility in the CER period (Y_{CER}) for the first FHPE step, indicating the non-saturation of the solvent for high flow rates. For the

second FHPE step, the extract solubility from a co-extracted raw material was almost independent ($1.00\text{-}1.31 \times 10^{-4}$, w/w) of the solvent flow rate, that showed the solvent selectivity in the process. Comparing to non fractionated ethanolic extractions using similar solvent flow rates ($\sim 8\text{-}21 \times 10^{-5}$ kg/s), the presence of different substances in the raw material led to higher solubilities ($1.53\text{-}4.11 \times 10^{-4}$, w/w), that were also influenced by the CO_2/EtOH flow rate.

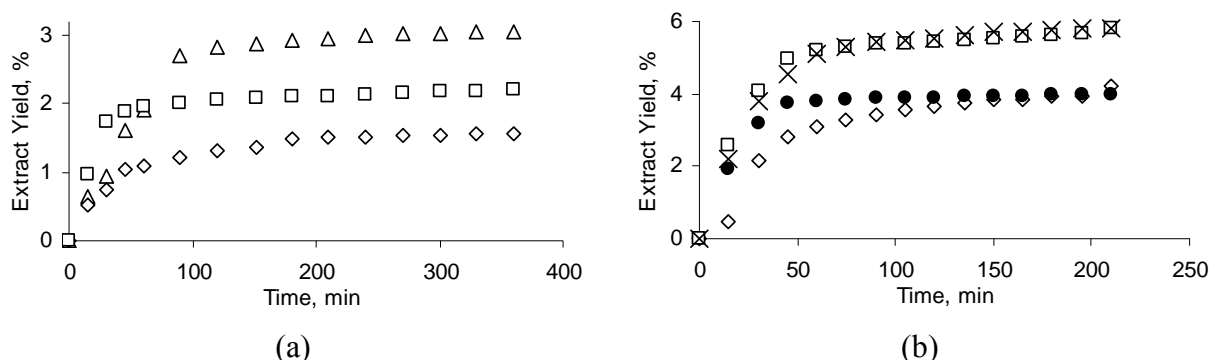


Figure 1: HPE pine bark kinetics at 30 °C and 25 MPa: (a) Solvent - CO_2 + 10 % EtOH; flow rate: $\Delta \sim 8 \times 10^{-5}$, $\diamond \sim 14 \times 10^{-5}$ and $\square \sim 21 \times 10^{-5}$ kg/s; (b) Flow rate - $\sim 8 \times 10^{-5}$ kg/s; solvent - CO_2 + EtOH in the following percentages (v/v): \diamond 30 %, \bullet 50 %, \square 70 %, \times 90 %.

Table 2: HPE and FHPE kinetic parameters and global yields, and total phenols (expressed as gallic acid equivalents) in ethanolic extracts obtained by high pressure methodologies and SoE.

Extraction Methodology	$M_{\text{CER}} \times 10^8$ (kg/s)	$Y_{\text{CER}} \times 10^4$	$t_{\text{CER}} / 60$ (s)	R_{CER} (%, db)	Fitting error ¹	Global Yield (%, db)	Gallic Acid Eq. (%,db)
FHPE-1 st step $\sim 7 \times 10^{-5}$ kg/s	1.75	2.53	24	0.38	0.01	0.99	-
FHPE-2 nd step $\sim 8 \times 10^{-5}$ kg/s	0.80	1.00	106	0.79	0.11	1.20	1.71
FHPE-1 st step $\sim 12 \times 10^{-5}$ kg/s	1.74	1.40	22	0.39	0.02	0.55	-
FHPE-2 nd step $\sim 12 \times 10^{-5}$ kg/s	1.54	1.27	16	0.25	0.01	0.73	1.87
FHPE-1 st step $\sim 17 \times 10^{-5}$ kg/s	0.78	0.45	32	0.25	0.05	0.61	-
FHPE-2 nd step $\sim 18 \times 10^{-5}$ kg/s	2.39	1.31	16	0.39	0.02	0.64	1.16
HPE $\sim 8 \times 10^{-5}$ kg/s	3.17	4.11	85	2.7	0.26	3.08	0.10
HPE $\sim 14 \times 10^{-5}$ kg/s	2.14	1.53	51	1.1	0.31	1.57	0.64
HPE $\sim 21 \times 10^{-5}$ kg/s	5.64	2.71	33	1.9	0.12	2.20	1.21
HPE – 30 % EtOH	6.51	8.24	49	3.3	0.61	4.48	1.51
HPE – 50 % EtOH	10.4	13.0	34	3.7	0.45	4.06	1.77
HPE – 70 % EtOH	15.9±3.4	22.1±5.2	37±1.5	5.7±1.2	0.71±0.03	6.51±1.2	1.36±0.005
HPE – 90 % EtOH	9.83	13.3	48	4.8	1.39	5.86	1.93
SoE (EtOH)	-	-	-	-	-	-	21.7±2.4

$$^1 \text{ Fitting error} = \frac{1}{N} \sqrt{\sum \left(\frac{y^{\text{calc}} - y^{\text{obs}}}{y^{\text{obs}}} \right)^2}$$

As a consequence of this kinetic parameters behaviour, extract yields during the CER period (R_{CER}) and global yields, also indicated in **Table 2**, changed with the solvent flow rate. In general, higher R_{CER} values and global yields were obtained with the lowest flow rate. The high duration of the CER period achieved in this condition can be a drawback, especially for the 2nd FHPE step, for which the increment in the solvent flow rate resulted in a decrease in t_{CER} from 106 to 16 s. This tendency was not so marked for HPE (t_{CER} decreased from 85 to 33 s).

Considering the solvent composition effect on M_{CER} and Y_{CER} obtained for HPE (with 7.6×10^{-5} kg/s CO_2 /EtOH flow), the increment in the EtOH percentage from 10 to 70 % had a positive influence on these kinetic parameters, may be due to the presence of a higher amount of the liquid solvent phase in the extraction cell. R_{CER} values and global yields followed the same behaviour, while t_{CER} values followed approximately the opposite behaviour. The choice of $CO_2 + EtOH$ 70 % for the solvent mixture seems to be the more appropriate to obtain a high amount of extract in the shortest time, from pine bark at 30 °C and ~25.4 MPa, at the employed flow rate.

Extract yield recovered in the glass flask, the trap and lines cleaning represented, in average, 94.4, 4.9 and 0.7 % of the total extract obtained, respectively.

Comparing 1st FHPE step (CO_2) yield with that of HD (0.010 ± 0.005 % yield), it can be concluded that high pressure extraction is an efficient process for pine bark oil extraction. Ethanolic SoE yield was 6.85 ± 0.4 %, close to the one achieved with HPE using 30 % CO_2 and 70 % EtOH.

TLC, performed for the analysis of low polarity compounds, confirmed that the fractioned extraction methodology succeeded in the obtention of extracts with diverse compositions: the 1st FHPE step extract was rich in volatile oil and the 2nd one in oleoresin. Extracts obtained with the non fractioned methodology ($CO_2 + EtOH$ 10 %) and 1st FHPE step extracts (CO_2) had similar composition in terms of low polarity compounds, and no variation with solvent flow rate was observed. When compared to the HD extract, these extracts were richer in substances with lower retention indexes. The substances presented in HPE extracts were also observed in the Soxhlet extract.

Total phenols in ethanolic extracts are also reported in **Table 2**. Even though flow rate had no positive impact on the extracts phenols amounts for the FHPE methodology, it had for HPE, for which the lowest flow rate resulted in the extract with the lowest total phenol content (0.10 % of gallic acid equivalents). Considering the HPE solvent composition effect, the EtOH percentage had a general positive effect on the amount of phenols in extracts, which should be associated with the presence of a higher amount of a high density and polarity phase that had a higher capacity of dissolving these polar substances. When compared to SoE extract (with 21.7 % of gallic acid equivalents), high pressure extracts can not compete in terms of phenolic contents, and so it can be concluded that the nature of the solvent and the high temperature used (EtOH boiling point) were more favourable to the extraction of phenols.

CONCLUSIONS

Solvent flow rate influenced high pressure extraction kinetics in a different way for fractioned and non-fractioned high pressure methodologies. Concerning Y_{CER} , non-saturation of the solvent was observed for high flow rates; concerning M_{CER} , higher flow rates favoured mass transfer for the ethanolic experiments. With respect to the solvent composition effect, Y_{CER} and M_{CER} increased with the increment in the EtOH percentage from 10 to 70 % that also increased global yields (3.08-6.51 %), and extract phenolic amounts (0.10-1.93%).

ACKNOWLEDEGMENTS

I. J. Seabra and M. E. M. Braga acknowledge Fundação para a Ciência e Tecnologia (FCT-MCTES) for the doctoral and postdoctoral fellowships, respectively (SFRH/BD/29133/2006 and SFRH/BPD/21076/2004).

REFERENCES

- [1] WOOD, J. E., SENTHILMOHAN, S. T., PESKIN, A. V., Food Chemistry, Vol. 77, **2002**, p. 155.
- [2] ROHDEWALD, P., Int. Journal of Clinical Pharmacology and Therapeutics, Vol. 40, **2002**, p. 158.
- [3] JACOBS, M., The chemical analysis of foods and food products. 3^a ed. Robert Krieger Publishing Co., **1973**, p.21.
- [4] SEABRA, I. J., BRAGA, M. E. M., BATISTA, M. T. P., SOUSA, H.,C., Extração fraccionada de taninos condensados de subprodutos de Pinus pinaster e Sambucus nigra L. Aplicaciones Industriales de los Taninos Vegetales. Eds. Cassel, E.; Vargas, M.F. E-book - CYTED and CNPq. ISBN 978-85-7430-674-2. Porto Alegre, Brazil, 2007.
- [5] Association of Official Analytical Chemists, A.O.A.C. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists: Arlington, **1984**, p. 1053.
- [6] WAGNER, H., BLADT, S., ZGAINSKI, E. M., Plant Drug Analysis. A Thin Layer Chromatography Atlas. Springer-Verlag: Berlin, Germany, **1984**.
- [7] SINGLETON, V. L., ROSSI, J. A. jr., American Journal of Enology and Viticulture, Vol. 16, **1965**, p. 144.
- [8] CHEUNG, L. M.; CHEUNG, P. C. K.; OOI, V. E. C., Food Chemistry, Vol. 81, **2003**, p. 249.
- [9] RODRIGUES, V. M.; SOUSA, E. M. B. D.; MONTEIRO, A. R.; CHIAVONE-FILHO, O.; MARQUES, M. O. M.; MEIRELES, M. A. A., Journal of Supercritical Fluids, Vol. 22, **2002**, p. 21.