Supercritical fluid extraction of triterpenic acids from *Eucalyptus globulus* bark at different scales

Marcelo M.R. de Melo[a], Rui M.A. Domingues[a], Martin Sova[b], Edward Lack[b], Helmut Seidlitz[b], F. Lang Jr.[b], Armando J.D. Silvestre[a], Carlos M. Silva*[a]

[a]*CICECO, Department of Chemistry, University of Aveiro, Aveiro, 3810-193, Portugal*
[b]*NATEX Prozesstechnologie GesmbH, Werkstrasse 7, 2630 Ternitz, Austria*

*Corresponding author: Tel.: + 351 234 401549; Fax: + 351 234 370084
E-mail address: carlos.manuel@ua.pt

ABSTRACT

Under the context of biorefinery, the supercritical fluid extraction (SFE) of *Eucalyptus globulus* bark has been investigated aiming at the production of extracts enriched in triterpenic acids (TTAs). Supported by previous studies of the optimization of the SFE operating conditions and subsequent modeling of extraction curves, the scale-up of the process was performed. Accordingly, cumulative curves were measured at three distinct scales (0.5, 5.0 and 80.0 L) for 200 bar, 40ºC and 2.5 wt.% ethanol. The flow rate to biomass weight ratio was fixed in 10 h^{-1} and was assumed as the scale-up criterion in view of the evidenced intraparticle diffusion resistance pointed by modelling results. The extraction yield and TTAs concentration results obtained for the three different scales evidenced good agreement, therefore confirming the validity of the adopted scale-up criterion and legitimating the technical viability of this process to be explored at commercial scale.

INTRODUCTION

Within the last years, the University of Aveiro has been studying fundamentals and applications of supercritical fluids, particularly transport properties [1-9], supercritical fluid extraction (SFE) [10-22], economic analysis [19, 21, 23], and general modeling and simulations [1-9, 13, 21, 24-25]. In this context, the SFE of the lipophilic [13, 15, 17, 20, 26] and phenolic [22] fractions of *Eucalyptus* bark, phenolics and oil from grape seed [11, 18, 23-24], diterpenes and oil from spent coffee grounds [14, 19] has been focused. Other species are presently under investigation.

The *E. globulus* bark is a major waste stream in the pulp and paper industry of countries such as Portugal, Spain, Brazil and Chile, and typically has no other application than being burned for power generation [20]. In this context, our research path sought to identify promising molecules in this biomass, and it was possible to signalize an interesting fraction of triterpenic acids (TTAs), such as ursolic, oleanolic and betulinic acids [27-30], with remarkable bioactivity properties [31].

Up to this point, the investigation on *E. globulus* bark has covered the following progressive stages: characterization of *Eucalyptus spp.* and respective content of their different morphological parts [27-30], preliminary study of SFE viability to extract TTAs [17], prediction of target compounds solubility and its impact on mass transfer fluxes [20], optimization of the SFE operating conditions (pressure, temperature, flow rate, cosolvent content) [15], and measurement and modeling of supercritical extraction curves to disclose dominant mass transfer mechanisms and thus establish a scale-up criterion [13].

In this sense, scale-up experiments carried out in the facilities of University of Aveiro and Natex [32] are now reported, being the objective of this communication the presentation of upscaling studies supported by previous results at lab scale. At the end, this work is intended to move a step forward on the demonstration of the technical proficiency of SFE to produce valuable natural extracts from a high volume / low vale agroforestry residue.

MATERIALS AND METHODS

Samples. Deciduous bark of *E. globulus* was randomly harvested from a 20-year-old clone plantation cultivated in Eixo (40º37'13.56''N, 8º34'08.43''W), region of Aveiro, Portugal. The bark was then dried in an oven at 40ºC during approximately 72 h, reaching final moisture content between 2 and 5 wt.%, milled to granulometry lower than 2 mm.
Supercritical fluid extraction. Assays were carried out in three different apparatus. Lab scale experiments were performed in a 0.5 L unit described elsewhere [15, 20]. The intermediate scale (5 L) and pilot scale (80 L) experiments were carried out at Natex facilities [32].
GC-MS analysis. The supercritical extracts were characterized by GC–MS. Samples of ca. 20 mg of each dried extract were trimethylsilylated according to the literature [27, 29]. Further details on the method and equipment can be found elsewhere [15, 20].

RESULTS

Optimization of operating conditions. As a preparation stage for the scale-up studies, one should recall the optimization study using Factorial Design of Experiments and Response Surface Methodology, that allowed trends on pressure, temperature and ethanol to be unveiled, and the optimum conditions that maximize total extraction yield (η_{total}) and TTAs concentration (x_{TTAs}) to be known. Pressure was found to positively influence both responses, while temperature exhibited the reverse effect. In addition, the inclusion of ethanol in concentrations up to 5 wt.% was shown to improve both total extraction yield and TTAs concentration in a degree comparable to the effect of doubling the pressure. The optima conditions (200 bar, 40ºC, and 5 wt.% ethanol) led to η_{total} = 1.2% (wt.) (against 1.3% by Soxhlet extraction using dichloromethane) and x_{TTAs} = 40.2% (wt.) (against 49.9% by Soxhlet) [15, 20]. Results from this study are graphed in Figure 1a. Afterwards, the solvent flow rate was also carefully studied, by increasing its value until no further enhancement of the extraction curves was observed, i.e. until the external film resistance was eliminated (see Figure 1b). As a result, a CO_2 flow rate of 12 g min^{-1} was found to be the most appropriate [13].

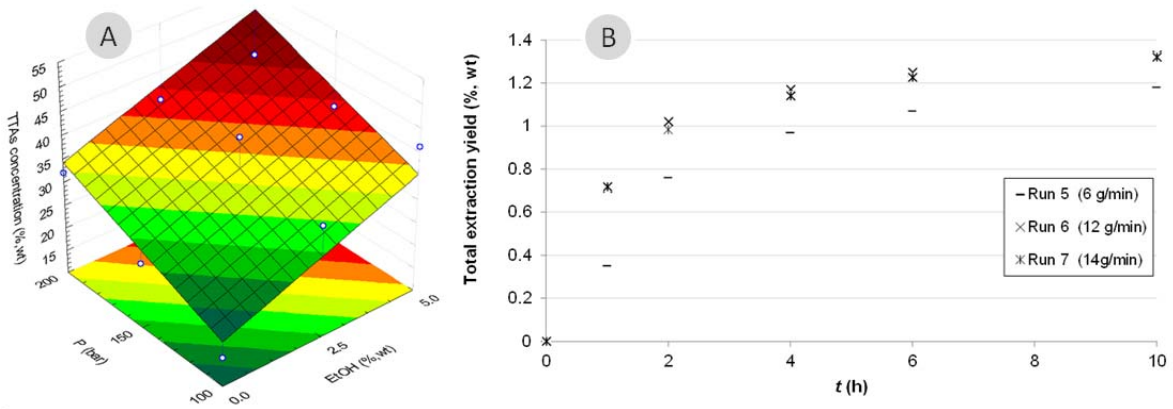

Figure 1– Supercritical CO_2 extraction of *E. globulus* peeling bark: a) TTAs concentration as function of pressure and temperature, plotted for a temperature of 40ºC [15]; b) total extraction yields *vs.* time curve, at 200 bar, 40ºC and 5 wt.% ethanol, for different CO_2 flow rates [13].

Modeling, and selection of scale-up criterion. As far as modeling is concerned, four simple and well known equations from the literature were fitted to the extraction curves, namely, the Logistic Model, Desorption Model, Simple Single Plate (SSPM) Model and Diffusion Model (DFM) [13, 21]. Figure 2 illustrates some results, being the best fittings achieved by DFM and SSPM, with average errors ranging 2.6-11.3%. The higher adequacy of these models pointed that intraparticle diffusion was the dominant mass transfer mechanism.

This insight was crucial for the definition of the scale criterion. Taking into consideration the dominant intraparticle diffusion limitations, the proposed scale-up criterion for SFE processes is the flow rate-to-biomass ratio [21, 33], which in this case establishes $Q_{CO2}\ w^{-1}_{biomass} = 10\ h^{-1}$ [16].

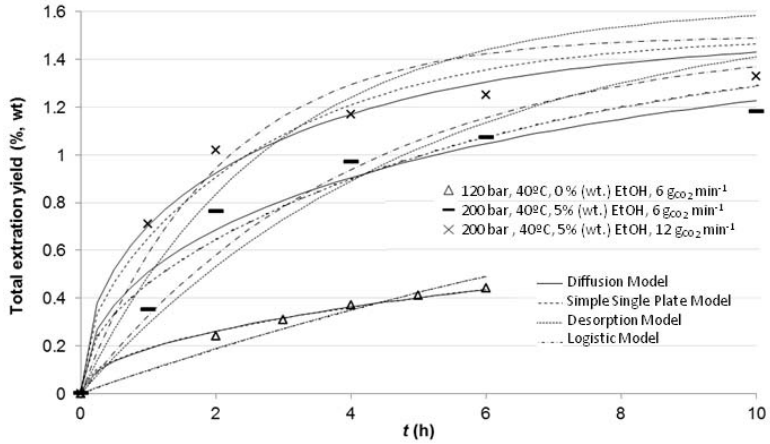

Figure 2 – Cumulative curves of total extraction yield of *E. globulus* bark at different pressure, temperature, ethanol content and flow rate. Lines represent modeling results. Plot adapted from [13].

Scale-up of the SFE process. In view of the promising results of SFE of *E. globulus* bark obtained at lab scale (0.5 L), it was decided to move forward to scale-up attempts, by performing experiments at two upper scales: 5 L and 80 L [16]. The following operating conditions were chosen: 200 bar, 40ºC, 2.5 wt.% ethanol, and the referred Q_{CO2} $w^{-1}_{biomass}$ ratio of 10 h^{-1} was ensured.

Figure 3 presents the cumulative curves for the SFE of *E. globulus* bark at pilot (80.0 L), intermediate (5.0 L) and lab (0.5 L) scales, being the results graphed in η_{total} *vs.* time and x_{TTAs} *vs.* time coordinates. Despite the total yield results were very close at the end of the extraction, differences were observed in the curvature region, with the runs for 5.0 and 80.0 L falling below the lab scale curve. This behaviour may be due to geometric differences between the extractors, as well as to the fact that SC-CO_2 is recycled in the two larger units. Similar trends were reported by other authors [34]. In the whole, the scale-up results were essentially concordant, taking into account that they were obtained using three units with distinct geometries.

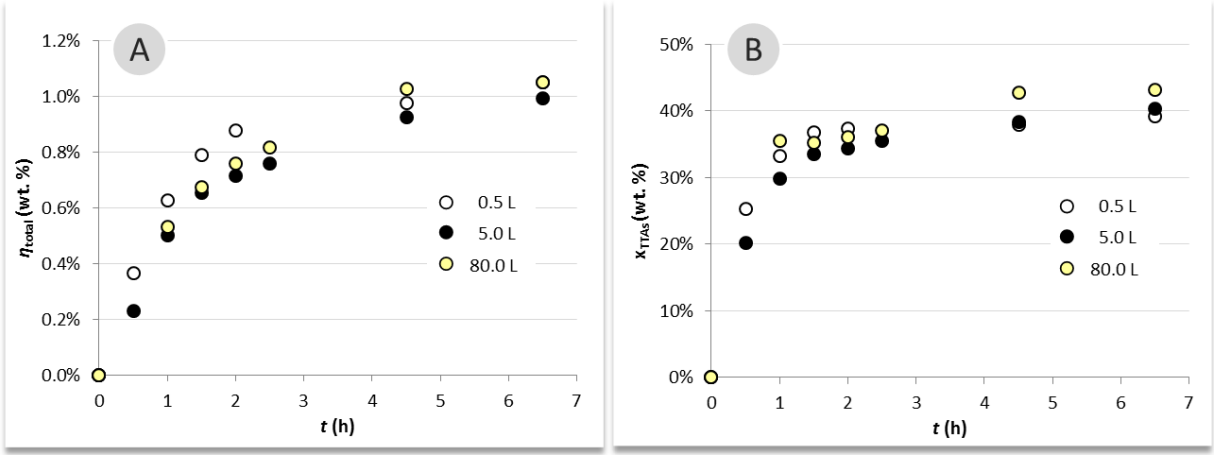

Figure 3 – Cumulative curves of (A) total extraction yield and (B) TTAs concentration for the SFE of *E. globulus* bark at pilot (80.0 L), intermediate (5.0 L) and lab (0.5 L) scales. Operating conditions of SC-CO_2 extraction: 200 bar, 40ºC, 2.5 wt% ethanol, Q_{CO2} $w^{-1}_{biomass}$ = 10 h^{-1}. Data taken from [16].

CONCLUSION

The SFE of *E. globulus* bark provides extracts with yields comparable to those obtained by conventional solid-liquid extraction using dichloromethane.

The variables that govern the SC-CO_2 extraction process are pressure, ethanol (cosolvent) content, and mass flow rate. Optimization of operating conditions at lab scale (0.5 L extractor) achieved 200 bar, 40ºC, 2.5 wt.% ethanol. From the experimental and modeling results, an appropriate scale-up criterion was established, i.e. $Q_{CO2}\ w^{-1}_{biomass} = 10\ h^{-1}$, and validated at higher scales, namely at 5.0 L and 80.0 L. The global yields and the TTAs concentration in the collected extracts measured for 0.5, 5.0 and 80.0 L are essentially concordant.

The SFE proficiently meets the scientific and technical challenges of adding value to this low-value / high volume residue of the pulp and paper industry, giving rise to interesting extracts for nutraceutical or cosmetic applications

AKNOWLEDGEMENTS

We acknowledge Associate Laboratory CICECO (Pest-C/CTM/LA0011/2013) and the 7th Framework Programme FP7/2007-2013 for funding project AFORE: Forest Biorefineries: Added-value from chemicals and polymers by new integrated separation, fractionation and upgrading technologies (CP-IP 228589-2).

REFERENCES

[1] C.M. Silva, H.Q. Liu, Modeling of Transport Properties of Hard Sphere Fluids and its Applications, in: A. Mulero (Ed.) Theory and Simulation of Hard Sphere Fluids and Related Systems, Lecture Notes in Physics 753, Springer, **2008**, pp. 383-501.

[2] P.F. Lito, A.L. Magalhães, J.R.B. Gomes, C.M. Silva, Universal model for accurate calculation of tracer diffusion coefficients in gas, liquid and supercritical systems, J. Chromatogr. A , 1290 (**2013**) 1-26.

[3] R.V. Vaz, A.L. Magalhães, D.L.A. Fernandes, C.M. Silva, Universal correlation of self-diffusion coefficients of model and real fluids based on residual entropy scaling law, Chem. Eng. Sci., 79 (**2012**) 153-162.

[4] A.L. Magalhães, F.A. Da Silva, C.M. Silva, Tracer diffusion coefficients of polar systems, Chem. Eng. Sci., 73 (**2012**) 151-168.

[5] A.L. Magalhães, P.F. Lito, F.A. Da Silva, C.M. Silva, Simple and accurate correlations for diffusion coefficients of solutes in liquids and supercritical fluids over wide ranges of temperature and density, J. Supercrit. Fluids, 76 (**2013**) 94-114.

[6] A.L. Magalhães, F.A. Da Silva, C.M. Silva, Free-volume model for the diffusion coefficients of solutes at infinite dilution in supercritical CO_2 and liquid H_2O, J. Supercrit. Fluids, 74 (**2013**) 89-104.

[7] A.L. Magalhães, R.V. Vaz, R.M.G. Gonçalves, F.A. Da Silva, C.M. Silva, Accurate hydrodynamic models for the prediction of tracer diffusivities in supercritical carbon dioxide, J. Supercrit. Fluids, 83 (**2013**) 15-27.

[8] R.V. Vaz, A.L. Magalhães, C.M. Silva, Prediction of binary diffusion coefficients in supercritical CO_2 with improved behavior near the critical point, J. Supercrit. Fluids, DOI: 10.1016/j.supflu.2014.1003.1011.

[9] E.L.G. Oliveira, A.J.D. Silvestre, C.M. Silva, Review of kinetic models for supercritical fluid extraction, Chemical Engineering Research and Design, 89 (**2011**) 1104-1117.

[10] M.M.R. de Melo, A.J.D. Silvestre, C.M. Silva, Enhanced technologies for the valorization of waste and side vegetable products using supercritical fluids, in: S. Pandalai (Ed.) Recent Research Developments in Chemical Engineering, Vol. 6, Transworld Research Network., **2014,** ISBN : 81-7895-190-8

[11] C.P. Passos, R.M. Silva, F.A. Da Silva, M.A. Coimbra, C.M. Silva, Enhancement of the supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed, J. Supercrit. Fluids, 48 (**2009**) 225-229.

[12] M.M.R. de Melo, R.M.A. Domingues, A.J.D. Silvestre, C.M. Silva, Extraction and purification of triterpenoids using supercritical fluids: from lab to exploitation, Mini-Rev. Org Chem, (**2014**) DOI: 10.2174/1570193X113106660002.

[13] R.M.A. Domingues, M.M.R. de Melo, C.P. Neto, A.J.D. Silvestre, C.M. Silva, Measurement and modeling of supercritical fluid extraction curves of *Eucalyptus globulus* bark: Influence of the operating conditions upon yields and extract composition, J. Supercrit. Fluids, 72 (**2012**) 176-185.

[14] H.M.A. Barbosa, M.M.R. de Melo, M.A. Coimbra, C.P. Passos, C.M. Silva, Optimization of the supercritical fluid coextraction of oil and diterpenes from spent coffee grounds using experimental design and response surface methodology, J. Supercrit. Fluids, 85 (**2014**) 165-172.

[15] R.M.A. Domingues, M.M.R. de Melo, E.L.G. Oliveira, C.P. Neto, A.J.D. Silvestre, C.M. Silva, Optimization of the supercritical fluid extraction of triterpenic acids from *Eucalyptus globulus* bark using experimental design, J. Supercrit. Fluids, 74 (**2013**) 105-114.
[16] M.M.R. de Melo, R.M.A. Domingues, M. Sova, E. Lack, H. Seidlitz, F. Lang Jr., A.J.D. Silvestre, C.M. Silva, Scale-up of the supercritical fluid extraction of triterpenic acids from *Eucalyptus globulus* bark, J. Supercrit. Fluids, (**2014, submitted**)
[17] R.M.A. Domingues, E.L.G. Oliveira, C.S.R. Freire, R.M. Couto, P.C. Simões, C.P. Neto, A.J.D. Silvestre, C.M. Silva, Supercritical fluid extraction of *Eucalyptus globulus* bark – a promising approach for triterpenoids production, Int. J. Mol. Sci., *13(6),* (**2012**) *7648-7662*.
[18] C.P. Passos, R.M. Silva, F.A. Da Silva, M.A. Coimbra, C.M. Silva, Supercritical fluid extraction of grape seed (Vitis vinifera L.) oil. Effect of the operating conditions upon oil composition and antioxidant capacity, Chem. Eng. J., 160 (**2010**) 634-640.
[19] M.M.R. de Melo, H.M.A. Barbosa, C.P. Passos, C.M. Silva, Supercritical fluid extraction of spent coffee grounds: Measurement of extraction curves, oil characterization and economic analysis, J. Supercrit. Fluids, 86 (**2014**) 150-159.
[20] M.M.R. de Melo, E.L.G. Oliveira, A.J.D. Silvestre, C.M. Silva, Supercritical fluid extraction of triterpenic acids from Eucalyptus globulus bark, J. Supercrit. Fluids, 70 (**2012**) 137-145.
[21] M.M.R. de Melo, A.J.D. Silvestre, C.M. Silva, Supercritical fluid extraction of vegetable matrices: applications, trends and future perspectives of a convincing green technology, J. Supercrit. Fluids, (**2014, in press**) DOI: 10.1016/j.supflu.2014.04.007
[22] S.A.O. Santos, J.J. Villaverde, C.M. Silva, C.P. Neto, A.J.D. Silvestre, Supercritical fluid extraction of phenolic compounds from *Eucalyptus globulus* Labill bark, J. Supercrit. Fluids, 71 (**2012**) 71-79.
[23] M.M.R. de Melo, C.M. Silva, Supercritical fluid extraction of oil and bioactive compounds from grape residues: experimental optimization, modeling and economic evaluation, in: J.S. Câmara (Ed.) Grapes: Production, Phenolic Composition and Potential Biomedical Effects, Nova Publisher (**2014**)
[24] C.P. Passos, M.A. Coimbra, F.A. Da Silva, C.M. Silva, Modelling the supercritical fluid extraction of edible oils and analysis of the effect of enzymatic pre-treatments of seed upon model parameters, Chem. Eng. Res. Des, 89 (**2011**) 1118-1125.
[25] C.M. Silva; C.P. Passos; M.A. Coimbra; F.A. Da Silva, Numerical Simulation of Supercritical Extraction Processes. Chemical Product and Process Modeling (**2009**) Vol. 4: Issue 4, Article 9
[26] D.J.S. Patinha, R.M.A. Domingues, J.J. Villaverde, A.M.S. Silva, C.M. Silva, C.S.R. Freire, C.P. Neto, A.J.D. Silvestre, Lipophilic extractives from the bark of *Eucalyptus grandis* x *globulus*, a rich source of methyl morolate: selective extraction with supercritical CO_2, Ind. Crop. Prod., 43 (**2013**) 340-348.
[27] C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, J.A.S. Cavaleiro, Lipophilic extractives of the inner and outer barks of *Eucalyptus globulus*, Holzforschung, 56 (**2002**) 372-379.
[28] R.M.A. Domingues, D.J.S. Patinha, G.D.A. Sousa, J.J. Villaverde, C.M. Silva, C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, *Eucalyptus* biomass residues from agro-forest and pulping industries as sources of high-value triterpenic compounds, Cell. Chem. Technol., 45 (**2011**) 475-481.

[29] R.M.A. Domingues, G.D.A. Sousa, C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, *Eucalyptus globulus* biomass residues from pulping industry as a source of high value triterpenic compounds, Ind. Crop. Prod., 31 (**2010**) 65-70.
[30] R.M.A. Domingues, G.D.A. Sousa, C.M. Silva, C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, High value triterpenic compounds from the outer barks of several *Eucalyptus* species cultivated in Brazil and in Portugal, Ind. Crop. Prod., 33 (**2011**) 158-164.
[31] R.M.A. Domingues, A.R. Guerra, M. Duarte, C.S.R. Freire, C.P. Neto, C.M. Silva, A.J.D. Silvestre, Bioactive triterpenic acids: from agroforestry biomass residues, to promising therapeutic tools, Mini-Rev. Org Chem, (**2014**) DOI: 10.2174/1570193X113106660001.
[32] Natex, Extraction Plants,http://www.natex.at/, Accessed in: April 2014
[33] M. Perrut, Supercritical Fluid Applications: Industrial Developments and Economic Issues, Ind. Eng. Chem. Res., 39 (**2000**) 4531-4535.
[34] J.M. del Valle, O. Rivera, M. Mattea, L. Ruetsch, J. Daghero, A. Flores, Supercritical CO_2 processing of pretreated rosehip seeds: effect of process scale on oil extraction kinetics, J. Supercrit. Fluids, 31 (**2004**) 159-174.