J. of Supercritical Fluids 97 (2015) 247–255

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

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

Prospective pathway for a green and enhanced friedelin production through supercritical fluid extraction of *Quercus cerris* cork

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form
11 December 2014
Accepted 11 December 2014
Available online 20 December 2014

Keywords:
Cork
Friedelin
Modeling
Quercus cerris
Supercritical fluid extraction

ABSTRACT

Supercritical fluid extraction (SFE) was applied for the first time to *Quercus cerris* cork, and compared with Soxhlet with dichloromethane (DCM). Novel triterpenes, viz. betulin and squalene, and β-sitosterol were identified for the first time in the lipophilic extracts.

The SFE at 300 bar and 40–80 °C provided extracts much richer in friedelin–the major compound from both SFE and DCM extracts – with concentrations up to 40.6 wt%, against 26.0 wt% for DCM. The SFE yields ranged between 0.97 and 1.81 wt% with pure CO_2, and attained 2.83 wt% when ethanol was introduced as cosolvent (10 wt%). In this case, however, the friedelin concentration dropped significantly due to the additional removal of non-target compounds. In general, the experimental data and their trends were in accordance with the theoretical predictions of kinetic and equilibrium properties estimated in this work for the friedelin/SC-CO_2 system.

This study demonstrates that the SFE of *Q. cerris* cork arises as a prospective pathway for a green and enhanced friedelin production process under the biorefinery concept.

1. Introduction

Cork is one of the plant tissues forming the barks of some trees species. A great popularity of this natural material is already credited to the cork oak (*Quercus suber* L.), whose transformation is responsible for a myriad a products such as cork stoppers, insulation, surfacing and paneling materials, engine joints, etc [1]. Nevertheless, the Turkey oak (*Quercus cerris*) is also a potential important provider of cork that can be found in regions such as Eastern Europe and Minor Asia [2]. Since in countries like Turkey, the bark of *Q. cerris* is not used except for fuel, the extraction of chemicals from this forest residue may offer opportunities towards its integrated utilization under the biorefinery concept.

Within oak species, the barks vary in terms of cork proportion and spatial distribution. In the cork oak, the cork producing cells (the phellogen or cork cambium) make up a cylindrical continuous envelope of the stem and branches and are active through the tree's lifetime, making up a thick layer of cork [3,4]. In most tree species the phellogen is discontinuous and short-lived, being replaced at intervals by a new phellogen in an inner part of the bark, giving rise to a layered rhytidome-type bark structure. This is the case of the Turkey oak (*Q. cerris*) [3,4]. As cork has a rather unique set of properties and offers many utilization possibilities, the study of cork proportion in the *Q. cerris* rhytidome has recently raised interest. Chemical summative characterization of *Q. cerris* cork showed lesser suberin and higher lignin quantities than those of *Q. suber* [2]. The extractives contents were similar in both but the non-polar fraction was up to 11 wt% in *Q. cerris* cork [2] while in *Q. suber* cork it is around 3.6–6.0 wt% [5,6].

In the last decade the supercritical carbon dioxide (SC-CO_2) has gained importance for the extraction of chemicals from lignocellulosic materials [7–10]. The supercritical fluid extraction (SFE) is an intensively studied separation technology that commonly allows a selective removal of compounds from complex mixtures and vegetable matrices [8,10]. One may cite, for example, the extraction of triterpenoids from *Eucalyptus globulus* bark [11,12], whose bioactive properties have been demonstrated [13], and the SFE of spent coffee residues, which gives rise to extracts enriched in diterpenes like cafestol and kahweol [14].

When the target solutes are polar, SC-CO_2 can sometimes be tuned through the addition of a third party polar compound to the process, which acts as a cosolvent. In this respect, ethanol is

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http://dx.doi.org/10.1016/j.supflu.2014.12.008
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Nomenclature

Abbreviations

AR	aromatic
C_p	heat capacity
D_{12}	tracer diffusion coefficient
DCM	dichloromethane
FA	fatty acid
GC–MS	gas chromatography–mass spectrometry
$k_{f,i}$	convective mass transfer coefficient
LCAA	long chain aliphatic alcohol
M	molecular weight
P	pressure
POL	polyol
$\Re$	gas constant
Rt	retention time
S	entropy
SC-CO_2	supercritical carbon dioxide
SFE	supercritical fluid extraction
ST	phytosterol
tr.	traces
T	temperature
TT	triterpene
V	molar volume
y	solubility
w	Pitzer acentric factor
Z	compressibility factor
w_{cork}	mass of cork

Greek letters

ϕ	fugacity coefficient
η_t	total extraction yield
$\eta_{friedelin}$	friedelin extraction yield
μ	viscosity
ρ	density

Subscript

b	boiling
c	critical
CO_2	relative to carbon dioxide
bp	at normal boiling point
i	relative to species i; friedelin
m	melting

Superscript

*	equilibrium
sat	saturation
SCF	supercritical fluid

the most used cosolvent in SFE research [8] and is able to compensate the rather low polarity of CO_2. The SFE works on grapes [15], strawberries [16], and red pepper [17] are examples of such cosolvent usage.

While no SFE results have yet been published for *Q. cerris*, the reported extraction yields of both SFE and Soxhlet extraction with dichloromethane (DCM) of *Q. suber* cork revealed that friedelin is the main component of both extracts with concentrations around 20 wt%, among five other triterpenes detected by ^{13}C NMR [5]. In addition Sousa et al. [6] identified cerine, friedelin and betulinic acid as major compounds in DCM-extracted natural and boiled corks. These findings induced us to investigate the potential of *Q. cerris* under the context of green solvents like SC-CO_2, with a particular attention to triterpenic compounds. Accordingly, the aim of the this study was to report for the first time the SFE of *Q. cerris* cork, with the respective composition of extracts and the impact of operating conditions on extraction yields and composition. These results are compared and contrasted with available SFE and Soxhlet results of *Q. suber* cork [5,6,18].

Concerning the structure of the article, Section 2 is devoted to "Materials and methods", followed by the "Results and discussion" part (Section 3), which is subdivided into: Section 3.1, for the assessment of Soxhlet extraction results in terms of yield and composition; Section 3.2 for the theoretical study of how kinetic and equilibrium properties of the SC-CO_2 and friedelin evolve with temperature; and finally Section 3.3, for the analysis of the SFE results in terms of yield and friedelin concentration. The conclusions of the work are drawn in Section 4.

2. Materials and methods

2.1. Chemicals and plant material

Nonacosan-1-ol (98% purity) and β-sitosterol (99% purity) were purchased from Fluka Chemie (Madrid, Spain); ursolic acid (98% purity), betulinic acid (98% purity), and oleanolic acid (98% purity) were purchased from Aktin Chemicals (Chengdu, China); betulonic acid (95% purity) was purchased from CHEMOS GmbH (Regenstauf, Germany); palmitic acid (99% purity), dichloromethane (99% purity), pyridine (99% purity), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (99% purity), trimethylchlorosilane (99% purity), and tetracosane (99% purity) were supplied by Sigma Chemical Co. (Madrid, Spain). CO_2 (99.95%) was supplied by Praxair (Porto, Portugal).

Q. cerris bark was obtained from Kahramanmaras, Turkey, and was granulated with a hammer-type industrial mill. The resulting granules were separated by density difference in distilled water in 10 min mixing time. The floating fraction of cork-enriched granules (subsequently named cork) was dried and grinded into 20–40 mesh (0.42–0.84 mm).

2.2. Soxhlet and supercritical fluid extractions

Soxhlet extractions were carried out with DCM (120 mL) using 1 g of 20–40 mesh *Q. cerris* cork during 8 h. The SFE experiments were performed at constant pressure (300 bar) and CO_2 flow rate ($11.0\,g_{CO_2}\,min^{-1}$). All operating conditions may be found in Table 1: pressure (P), temperature (T), extraction time (t), mass of cork (w_{cork}), and ethanol concentration. The influence of temperature (40–80 °C) and ethanol content (0 and 5 wt%) upon total extraction yield (η_t) and extract composition were evaluated. One experiment was conducted sequentially with the same biomass in four successive steps (SFE1.1, SFE1.2, SFE1.3 and SFE1.4; see Table 1) in order to disclose the impact of varying temperature and ethanol concentration.

2.3. Analyses of extracts by GC–MS

The composition of the SFE and Soxhlet extracts was determined by gas chromatography–mass spectrometry (GC–MS). Approximately 20 mg of dried extract were converted into trimethylsilyl (TMS) derivatives according to the literature [19]. Tetracosane was used as internal standard, and the calibration of the equipment for the various families of compounds was performed using nonacosan-1-ol, β-sitosterol, ursolic acid, betulinic acid, oleanolic acid, betulonic acid, and palmitic acid.

The analyses were performed using a Trace Gas Chromatograph 2000 Series equipped with a Thermo Scientific DSQ II mass spectrometer, using helium as carrier gas ($35\,cm\,s^{-1}$), and a DB-1 J&W capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness).