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Enhancement of the supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed

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ABSTRACT

The supercritical fluid extraction of grape seed (*Vitis vinifera* L.) oil using carbon dioxide has been carried out at constant temperature (313.15 K) and solvent flow rate (1.7×10^{-4} kg s^{-1}), at 160, 180 and 200 bar, using both untreated and enzymatically pre-treated seeds. The pre-treatment of triturated seeds has been performed with a cell wall degrading enzyme cocktail containing cellulase, protease, xylanase, and pectinase, in order to enlarge the broken/intact cells ratio, thus increasing oil availability. The maximum extraction yield obtained was 16.5%, which is 44% higher than the 11.5% yield obtained with untreated seeds.

The cumulative extraction curves measured show two characteristic periods: a first linear part where the majority of the oil is obtained, and a second asymptotic branch which contributes with only 3–8% to the total oil removed.

As pressure rises, the mass of CO_2 needed to reach a definite extraction yield decreases and the linear part of the extraction curves of treated and untreated seed approach themselves.

1. Introduction

Grape seed (*Vitis vinifera* L.) is a well known oilseed crop containing typically 8–15% (w/w) of oil with recognised quality due to its high level of unsaturated fatty acids, namely oleic and linoleic [1] and antioxidant-rich compounds. In terms of applications, it is becoming increasingly popular for culinary, pharmaceutical, cosmetics, and medical purposes [2]. It is also an appealing product due to its large availability as a major by-product of wine industry [3,4]. Grapes give origin to approximately 25% (w/w) of dry pomace, of which one third is seed [5]. Hence, despite winemaking residues have been considered traditionally an economic and environmental problem, they are now becoming increasingly recognised as valuable commodities for the production of added value products.

Industrially, the process commonly adopted to obtain edible oil from vegetable seeds foresees several stages: clean up of the biomass, drying, crushing, and pressing. During pressing most of the oil is extracted from the seeds, but a considerable amount remains in the final cake. This is then extracted with *n*-hexane which is evaporated afterwards. Finally, if necessary, the oil has to be refined to fulfil the requirements for human consumption [6]. One obvious drawback of this process, that may inclusively dictate its future viability, is the utilization of *n*-hexane in last stages. In fact, even seeds coming from biological agriculture loose their biological status and, consequently, the extracted oil too. Supercritical (SC) carbon dioxide emerges as a suitable solvent to produce these oils.

The use of supercritical fluids (SCF) has been attracting widespread interest owing to their unique properties (e.g., liquid-like solvent power, negligible surface tension, and gaslike transport properties), versatile applications, and changes in environmental regulations which foster the utilization of green solvents. In this field, carbon dioxide has been especially adopted since it is essentially non-toxic, non-flammable, inexpensive, can be recycled, is totally dissipated from extracts at atmospheric pressure, and has easily accessible critical conditions [7,8].

The supercritical fluid extraction (SFE) of grape seed oil has proven to reach extraction yields equivalent to those achieved by conventional Soxhlet with *n*-hexane [3].

The semi-continuous SFE may be characterised by extraction curves, a plot of the accumulated amount of extract against time or, equivalently, against the amount of solvent passed through the extractor [4]. They usually comprise two extraction periods, a first one characterised by a rapid oil extraction followed by a period of slow to very slow additional oil yield. This represents a two-mechanism extraction process where a rapid extraction of sur-

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Nomenclature

D_{12}	tracer diffusion coefficient
FPR	forward pressure regulator
k_c	convective mass transfer coefficient
m	mass
$\dot{m}$	mass flow
MFM	mass flow meter
NV	needle valve
P	pressure
Re	Reynolds number
Sc	Schmidt number
Sh	Sherwood number
T	temperature
Y	oil solubility

Greek letters

η	extraction yield (%, w/w)
μ	viscosity
ρ	density

face and shallow subsurface oil is followed by diffusion-controlled extraction of the more deeply embedded oil [9]. With respect to modelling, the sudden reduction of the extraction rate cited above was taken into account introducing the concept of broken and intact cells [10]. The first ones prevail close to the surface, where cell walls have been damaged by pre-treatment processes, while the particle core contains intact cells. Mass transfer resistance through cell walls is high, which justifies the large difference found between the transfer rates of both regions [4].

In this work, the extraction of grape seed oil has been carried out with supercritical CO_2. Moreover, the effect of an enzymatic pre-treatment of the seed upon the process yield and the cumulative curves has been also investigated. The pre-treatment has been accomplished using a cell wall degrading enzyme cocktail with pectinase, xylanase, protease, and cellulase activities. The choice upon the type of enzymes used was based on the knowledge that the oil removal can be favoured upon partial hydrolysis of the plant cell walls by means of appropriate enzymes [11], using the best operating conditions previously assessed [12].

2. Experimental

2.1. Materials and reagents

Seeds were collected from grapes (*Vitis vinifera* L.) of the red variety 'Touriga Nacional' harvested for red wine manufacture in Bairrada Appellation (Anadia, Portugal) during September 2007. Cellulase produced from *Aspergillus niger* (commercial code no. 22178), hemicellulase produced from *Thermomyces lanuginosus* (commercial code no. X2753), pectinase produced from *Aspergillus aculeatus* (commercial code no. P2611), and protease produced from porcine pancreas (commercial code no. 93614) have been purchased from Fluka Sigma–Aldrich Co. (St. Louis, MO). Other reagents were of analytical grade or higher available purity.

2.2. Seed preparation and enzymatic pre-treatment

2.2.1. Seed preparation, size reduction and screening

Seeds were collected during transfer of the musts in wine fermentation, and separated from pulp and skins by decantation and sieving. A first wash removed immature grains floating at water surface. Subsequently, the seeds were submitted to several washes with water (200 g/L) under gentle stirring with a magnetic bar at 4 °C during a minimum of 3 days, with two water exchanges per day, until a minimum constant turbidity was observed. The purified seeds were finally washed with ethanol, air dried at room temperature, and stored at 4 °C until use. Finally, milling was carried out on a domestic coffee mill, and the particles were classified in a standard sifter with several mesh sizes. For the experiments performed, only particles with an average size of 0.75 were selected.

2.2.2. Enzymatic pre-treatment

Prior to the SFE of grape seed oil, an enzymatic pre-treatment was performed based on a previous research [12]. The grape seeds have been treated with a cocktail of cellulase = 29, protease = 1191, xylanase = 21, and pectinase = 569 U/g seed sample, which have been added to flasks containing 40 mL of distilled water + milled seed. The enzymatic suspension to seed ratio has been kept equal to 4 L/kg through all experiments. The pH 4 has been fixed with a buffer solution of citric acid and sodium hydrogenphosphate. The reaction proceeded isothermally at 40 °C under continuous stirring at 200 rpm during 24 h, and was stopped by freezing the suspension with liquid nitrogen. Then, the water was removed by freeze-drying the content of the flasks.

The above-mentioned operating conditions were fixed after analysing the effect of each parameter upon the enzymatic activity, in an attempt to increase grape seed oil availability [12]. Carrying out Soxhlet measurements it was shown that the extraction yield increases with both increasing enzymes concentration and treatment time, whereas pH and temperature give rise to opposite behaviours. GC-FID analysis were performed to evaluate oil content, and allowed the authors to confirm that the global increment observed was due to triacylglycerides only.

2.3. Supercritical fluid extraction

2.3.1. Equipment

The SFE experiments were carried out with carbon dioxide under semi-continuous operation in an apparatus built/assembled at the University of Aveiro. A simplified scheme of the equipment is given in Fig. 1. The extraction chamber is cylindrical, stainless steel, with 1.6×10^{-4} m^3 (length H = 0.13 m, internal diameter i.d. = 0.04 m). The CO_2 withdrawn from a container is firstly liquefied in a refrigerated bath, to approximately 265 K, and then pressurised by an air driven liquid pump to a high-pressure vessel. The pressure inside the extractor is regulated via the forward pressure regulator FPR1. The mass flow meter (MFM) measures the instantaneous flow rate and total quantity of solvent delivered. The solvent is brought to the extraction temperature by means of a long tubing coil placed inside the oven. After percolating the seed bed, the extract stream passes through the forward pressure regulator FPR2 and a micrometering valve (NV), reaching atmospheric pressure; NV is used to control solvent flow rate. The FPR2 and the adjoining line are heated to prevent blocking up due to oil and CO_2 freezing. The recovery vessel at the exit has an internal volume of 3.0×10^{-4} m^3.

2.3.2. SFE experimental conditions and procedure

Approximately 0.07 kg of grape seeds previously prepared were charged into the extractor, and a small amount of steel shreds was packed at the top to prevent seed powder to escape. To analyse both the effect of the pressure (i.e., solvent density, ρ) and the enzymatic treatment upon the SFE, experiments were accomplished at P = 160, 180, and 200 bar, at constant temperature (T = 313.15 K) and carbon dioxide flow rate ($\dot{m}_{CO_2} = 1.7 \times 10^{-4}$ kg s^{-1}), for both untreated and pre-treated samples. The extraction curves were obtained by representing the yield or the quantity of extracted oil against consumed