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Supercritical fluid extraction of grape seed (*Vitis vinifera* L.) oil. Effect of the operating conditions upon oil composition and antioxidant capacity

Cláudia P. Passos[a], Rui M. Silva[a], Francisco A. Da Silva[a], Manuel A. Coimbra[b], Carlos M. Silva[a,*]

[a] CICECO, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[b] Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

In this work, the supercritical fluid extraction of grape seed oil has been studied at 180, 200 and 220 bar, and 313.15 and 323.15 K to analyse their influence upon oil quality.

The extraction curves exhibit an initial linear extraction period followed by a smooth asymptotic plateau at 11.5%. The extraction rate increases with increasing pressure and decreasing temperature, due to the influence of pressure and temperature upon the oil solubility; the convective mass transfer coefficient played a minor role on the process.

The triacylglycerides content and the fatty acids profile have been determined, being roughly unaffected by the operating conditions adopted. The antioxidant activity of the oil has been assessed by the DPPH• spectrophotometric method, and showed to increase with pressure and noticeably with temperature. Along extraction curves, the antioxidant activity is more pronounced on the oil collected during the first stages of the process, where 35–40% of total oil is extracted.

1. Introduction

Grape seed (*Vitis vinifera* L.) is a well known oilseed crop containing typically 8–20% (w/w) of oil [1,2], and 10–20% (w/w) of polyphenolic compounds in dry basis [3,4]. Hence, it is an appealing raw material due to its large availability as major by-product of the wine industry. Dry pomace accounts for, approximately, 25% (w/w) of grapes, of which about 20–26% (w/w) is seed [5,6]. Accordingly, despite winemaking residues have been considered traditionally an economic and environmental problem, they are becoming increasingly recognized as valuable commodities for the production of added value products.

The quality of grape seed oil is due to its high level of unsaturated fatty acids (ca. 90%), particularly linoleic (C18:2) and oleic (C18:1); traces of linolenic (C18:3) and palmitoleic (C16:1) may be also found [2,4]. On the other hand, the saturated fatty acids totalizes ca. 10%, which are mainly palmitic (16:0) and stearic (18:0). However, such high level of polyunsaturated fatty acids makes it extremely susceptible to oxidation [7].

The defatted seeds as a rich source of polyphenolic compounds have been largely studied by several research groups [3,8–12]. Frequently, water, methanol, ethanol, acetone, and ethyl acetate have been tested as extractants, and the resultant antioxidant activities of grape seed and skins are evaluated by, essentially, two major types of reactions: hydrogen atom transfer, and single electron transfer assays (e.g., Yilmaz and Toledo [12]; Díaz-Reinoso et al. [13]; Huang et al. [14]). Alternatively, in this work one focuses the supercritical fluid extraction (SFE) of grape seed oil and the determination of the antioxidant capacity (AOC) of the oil itself.

Unrefined oils may contain tocopherol and other bioactive compounds at concentrations around 0.02–1.2 g/kg, which in most applications are sufficient to provide the necessary antioxidant properties [4,15]. Nonetheless, such compounds are frequently lost during food processing, which demands mild extraction processes and operating conditions as well as higher extractability and selectivity [16]. Hence, the evaluation of the AOC of the extracted oil should be performed to tailor the extraction methods and operating conditions in order to preserve their bioavailability in the final product [13,16].

The industrial processes commonly adopted to produce edible oils from vegetable seeds comprehend several stages, where the extraction with *n*-hexane is an important one. However, this organic solvent is usually non-selective and accomplishes the simultaneous removal of non-volatile pigments and waxes, giving rise to dark coloured and viscous extracts contaminated with solvent residues. This renders them difficult to handle without further refining, and may inclusively dictate the future commercial viability of the oil. For instance, the introduction of organic solvents may even threat the biological status of oils obtained from seeds of biological agriculture [17].

* Corresponding author at: Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.
Tel.: +351 234 401549; fax: +351 234 370084.
E-mail address: carlos.manuel@ua.pt (C.M. Silva).

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Nomenclature

AOC	antioxidant capacity
Cn:m	fatty acid with n carbons and m double bounds
$C_{DPPH•}$	molar concentration of DPPH• free radical
$\bar{d}_p$	average particle diameter
d_{pi}	mesh size in standard sifter
DPPH•	1,1-diphenyl-2-picrylhydrazyl free radical
E_i	extract i
FAME	fatty acid methyl ester
FID	flame ionization detector
FPR	forward pressure regulator
GC	gas chromatography
GC–qMS	gas chromatography–quadrupole mass spectrometry
k_f	convective mass transfer coefficient
$\dot{m}_{CO_2}$	CO_2 mass flow rate
m_i	mass of solids retained below sieve i
m_t	total mass of solids classified in standard sifter
MFM	mass flow meter
P	pressure
RSC	radical scavenger capacity (Eq. (2))
SFE	supercritical fluid extraction
T	temperature
UV–vis	ultraviolet and visible absorption spectroscopy
Vn	valve n in the SFE equipment
y_s	oil solubility

Greek letters

η	extraction yield
ρ	CO_2 density
σ	standard deviation

The use of supercritical fluids as an alternative solvent for seed oil extraction has been attracting widespread interest owing to their particular properties (e.g., liquid-like solvent power, negligible surface tension, and gas-like transport properties), 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 at the industrial level, can be recycled, has easily accessible supercritical conditions, and is totally dissipated from extracts at atmospheric pressure avoiding the necessity of further expensive and harmful refining treatments [18,19]. Hence, the supercritical fluid extraction of grape seed oil is expected to preserve its natural phytochemicals, such as antioxidant tocopherols [13], ensuring the conservation of its high quality. Furthermore, it is worth noting the SFE has proven to reach extraction yields equivalent to those achieved by conventional Soxhlet with n-hexane, for which $\eta = 11.6\%$ [6,20].

This paper deals with the SFE of grape seed oil using CO_2, studying the effect that the operating conditions have on the quality of the final oil, as well as on the quality of the individual fractions collected during the extraction. The SFE was characterized by the corresponding extraction or cumulative curves, where the amount of extract obtained is plotted against time or, equivalently, solvent consumed. The triacylglycerides content, the fatty acids profile, and the antioxidant capacity of the oil samples were determined in order to provide information about the suitability of the processing conditions. The AOC was evaluated by measuring the total free radical scavenger capacity of the oil samples following the well established DPPH• method [21].

2. Experimental

2.1. Materials and reagents

Seeds were collected from grapes (*Vitis vinifera* L.) of the red variety *Touriga Nacional* harvested at technological maturity in Bairrada Appellation (Anadia, Portugal) during September 2007. Free stable DPPH radical (DPPH•), stearic acid, palmitic acid, methyl heptadecanoate, DL-α-tocopherol were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Fatty acids methyl esters (FAMEs) Mix C_8–C_{24}, was purchased from Supelco (Bellefonte, PA, USA). Commercial grape seed oil, obtained from cold pressing, was purchased from New Directions (Portugal). Other reagents were of analytical grade or higher available purity.

2.2. 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 kg m^{-3}) under gentle stirring, with a magnetic bar, at 277 K during a minimum of three days, with two water exchanges per day until a minimum constant turbidity has been observed. Then, they were washed with ethanol, air dried at room temperature, and stored at 277 K until use. Finally, milling was carried out on a domestic coffee mill, and the particles classified in a standard sifter with several mesh sizes (<0.5, 0.5–0.60, 0.60–0.71, 0.71–1.0, 1.0–1.4, 1.4–2.0, >2.0 mm). An average particle diameter $\bar{d}_p = 0.75$ mm was adopted, being calculated by Sauter's equation [22] to a set of fractions within the previous mesh sizes:

$$\bar{d}_p = \frac{m_t}{\sum_{i=1}^{k} m_i / d_{p_i}} \quad (1)$$

where m_i is the mass of particles retained below mesh size d_{p_i}, m_t is the total mass of milled seeds, and k is the number of mesh sizes.

2.3. Supercritical fluid extraction

2.3.1. Equipment

The SFE experiments were carried on 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 extractor is cylindrical, stainless steel made, with 1.6×10^{-4} m^3 (length $H = 0.13$ m, internal diameter ID = 0.04 m). The CO_2 withdrawn from a container is firstly liquefied in a refrigerated bath, where it is cooled to approximately 265 K, and then pressurized by an air driven liquid pump to a vessel. The pressure inside the extractor is regulated via the forward pressure regulator FPR1. The solvent is brought to the extraction temperature by means of a long tubing coil placed inside the oven (thermostatic casing). After percolating the seed bed, the extract stream passes through the forward pressure regulator FPR2, a cut valve (V4) and a micrometering valve (V5), reaching atmospheric pressure; V4 and V5 are 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 recovering vessel at the exit has an internal volume of 3.0×10^{-4} m^3. The mass flow meter (MFM) measures the instantaneous flow rate and total quantity of solvent delivered.

2.3.2. SFE experimental conditions and procedure

Approximately 0.07 kg of grape seeds previously prepared were loaded into the extractor, and a small amount of steel shreds was packed at the top to prevent seed powder to escape. The bed density and porosity were experimentally determined by gravimetry giving 688 kg m^{-3} and 0.37, respectively. SFE of grape seed

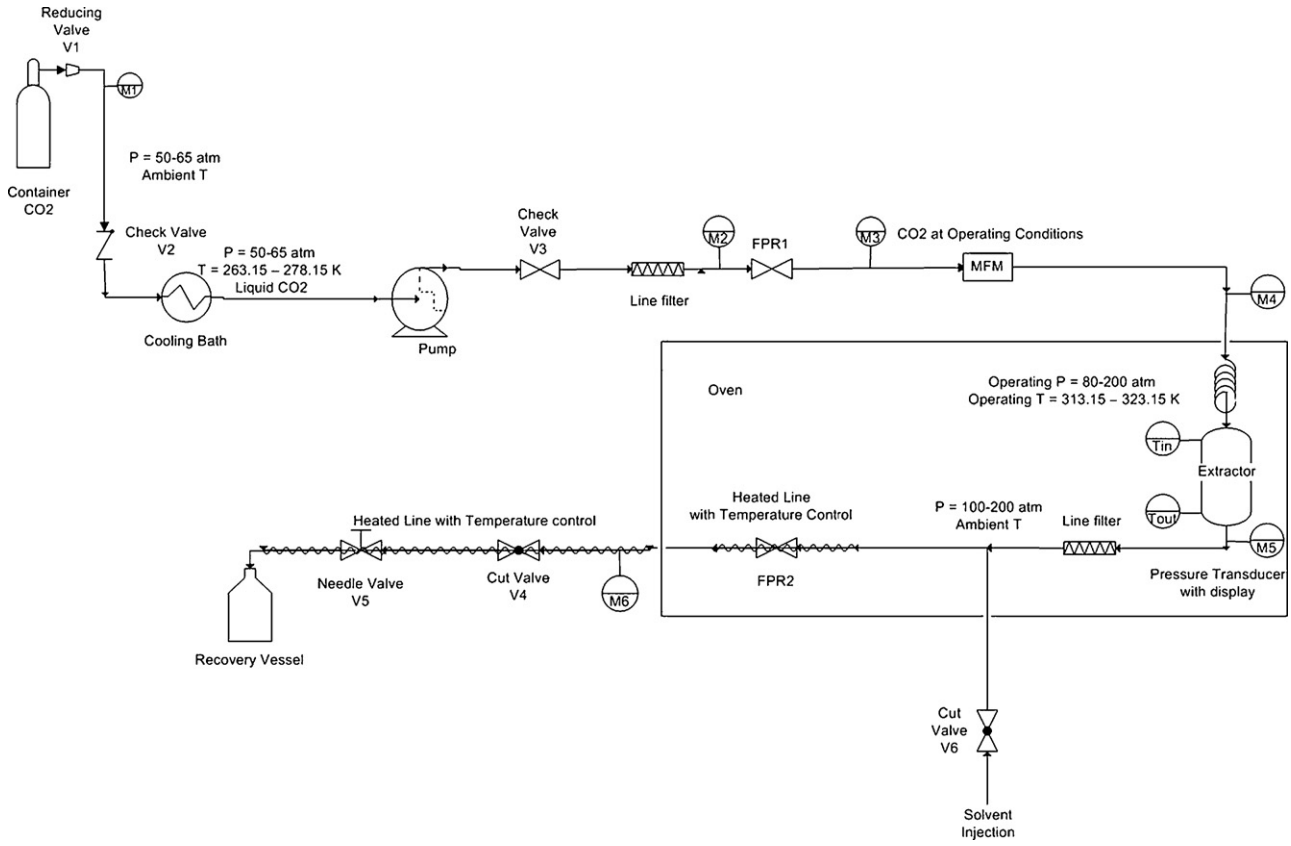

Fig. 1. Simplified scheme of the supercritical fluid extraction installation.

oil was accomplished at 180 bar/323.15 K, 200 bar/323.15 K, and 220 bar/313.15 K, at constant carbon dioxide flow rate $\dot{m}_{CO_2} = 1.7 \times 10^{-4}\,kg\,s^{-1}$. The results obtained in this work are compared with measurements available in a previous paper [20] for 180 bar/313.15 K and 200 bar/313.15 K, in order to analyse the effect of temperature. (Please note there is a mistake in the abscissas of the figures of Ref. [20]; they should be "kg of CO_2".) The extraction curves were obtained by representing yield (η) against consumed CO_2 (m_{CO_2}). The η (w/w) of the process is defined as the mass of extracted oil divided by the mass of dried seed loaded in the extractor. The extractor was operated discontinuously, for intervals of about 1 h, to assess several data points of the extraction curve. The oil precipitated along the pressure drop section (which comprehends the forward pressure regulator FPR2 and valves V4 and V5) was recovered washing with *n*-hexane, and then weighted. Each extraction curve presented in this paper is the result of two reproducible curves.

Conventional extraction was carried out for comparison, using 10 g of milled seed, and 150 cm^3 of *n*-hexane in a Soxhlet apparatus (50 cm^3 capacity; 23 mm × 100 mm cartridge) during 4 h.

2.4. Oil processing

The mass of extracted oil was determined gravimetrically after solvent evaporation. Furthermore, to ensure that the oil carries no water, the extracted samples were passed over sodium sulphate anhydrous under vacuum through a G1 sintered glass filter, and concentrated using a rotary evaporator at 303.15 K. The oil was then transferred to test tubes and dried by centrifugal evaporation (Univapo).

2.5. Tryacylglicerides profile

2.5.1. Transesterification

To characterize the extracted oil by gas chromatography, the FAMEs were prepared by transesterification with sodium methoxide, which was adopted since it is a very simple and fast methodology, and specially because it proceeds at ambient temperature, therefore the risk of decomposition of polyunsaturated fatty acids is reduced [23]. The procedure comprehends the preparation of an internal standard solution of heptadecanoate methyl ester with concentration 0.75 kg m^{-3}. The oil sample (0.100 g) was dissolved in 1 cm^3 of *n*-hexane, and mixed with 4 cm^3 of the internal standard solution. Then 0.2 cm^3 of a methanolic KOH solution (2 M) were added, the resulting sample was sealed and mixed vigorously for 30 s in a vortex shaker. After that 2 cm^3 of saturated sodium chloride solution were added, and the sample was submitted to a centrifugation at 2000 rpm during 5 min. Finally, 1 cm^3 of the organic phase was taken to a clean tube and aliquots (0.1–0.5 μL) were used for GC analysis.

2.5.2. GC-FID

Following the transesterification, the FAMEs were analysed and separated on a Gas Chromatograph (Perkin Elmer Clarus 400, USA), equipped with a 30 m × 0.32 mm (ID), 0.25 μm film thickness DB-FFAP fused silica capillary column (J&W Scientific Inc., Folsom, CA, USA) and a flame ionization detector. Split injection mode was used with a ratio of 20:1 (5 min). The GC injection port was programmed at 518 K, and the detector at 523 K. Oven temperature was programmed in three ramps, from 348 to 428 K at 15 K min^{-1}, from 428 to 453 K at 3 K min^{-1}, and from 453 to 493 K at 40 K min^{-1}, and held isothermal for 3 min. The carrier gas was hydrogen at 50 cm^3 min^{-1}.

The compounds were identified by comparing their retention times with those of a commercial FAMEs mixture (C_8–C_{24}).

2.5.3. GC–qMS

Besides GC-FID analyses, the samples of the global oil (see Table 2) were also analysed using a GC-qMS Agilent Technologies 6890N gas chromatograph, equipped with a 30 m × 0.32 mm (ID), 0.25 μm film thickness DB-FFAP fused silica capillary column (J&W Scientific Inc., Folsom, CA, USA), connected to an Agilent 5973 quadrupole mass selective detector. Spitless injection mode was used for 5 min. The oven temperature was programmed similarly to the GC-FID analyses above. The carrier gas was helium flowing at 1.7 $cm^3\ min^{-1}$. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV, scanning the range of 33–300 m/z in a 3 s cycle, in a full scan acquisition mode. Compounds' identification was accomplished by comparing GC retention times and mass spectra with those of standard substances. All mass spectra were also compared with the data system library (Wiley 275).

All measurements were run with at least three replicates, each one representing the analysis of one different aliquot (0.100 g) of grape seed oil. The reproducibility is represented with error bars in the corresponding figures.

2.6. Antioxidant capacity (AOC)

The antioxidant capacity of the oil samples was evaluated by the total free radical scavenger capacity (RSC) following the methodology described by Espín et al. [21]. Accordingly, the RSC is the variation of the concentration of DPPH• free radical, previously dissolved in ethyl acetate, after 60 min of reaction with the samples:

$$\mathrm{RSC} = C_{\mathrm{DPPH}^\bullet,i} - C_{\mathrm{DPPH}^\bullet,f} \quad (2)$$

where the initial and final concentrations are spectrophotometrically measured at 515 nm. The AOC of the grape seed oil extracted is then expressed in terms of tocopherol equivalents, i.e., the concentration of a tocopherol solution which gives rise to the same RSC.

A series of tocopherol standard solutions in ethyl acetate in the range of [0–6] × 10^{-3} M were prepared to build a calibration curve. Aliquots of 0.05 cm^3 of the previous solutions were added to 3.95 cm^3 of a DPPH• solution in ethyl acetate with concentration 1.5 × 10^{-4} M. The reactional mixture was shaken vigorously and allowed to react at room temperature in the dark. After 60 min, the concentration of the remaining DPPH• was determined colorimetrically at 515 nm, by blanking against an appropriate control (mixture without radical). A double beam ultraviolet–visible (UV/vis) spectrophotometer (Lambda 35, Perkin-Elmer, USA) was used to read samples absorbance. The calibration curve corresponded to the following relation expressed in molar concentrations:

$$\mathrm{AOC\,(mol\ tocopherol/dm^3)} = 43.4 \times \mathrm{RSC\,(mol\ DPPH^\bullet/dm^3)} \quad (3)$$

The RSC of grape seed oil was determined by weighing 0.05 mg of oil sample and adding directly to 3.95 cm^3 of a DPPH• solution in ethyl acetate with concentration 1.5 × 10^{-4} M. The oil was utilized without any pre-treatment.

The AOC of the oil is expressed as the concentration of an equivalent tocopherol solution which produces the same RSC. Thus, taking into account Eq. (3):

$$\mathrm{AOC} = C_{\mathrm{tocopherol}}(\mathrm{M}) = 43.4 \times \mathrm{RSC(M)} \quad (4)$$

Each result presents the mean and the standard deviation for a minimum of three experiments. Statistical analysis has been carried out using Student's t-test and outliers analyses [24]. Significance was defined at $p < 0.025$.

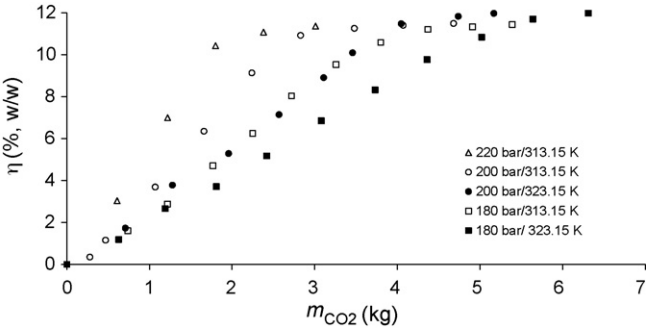

Fig. 2. Extraction curves for the SFE of grape seed oil.

3. Results and discussion

3.1. Supercritical fluid extraction curves

In Fig. 2 the cumulative extraction curves (η *versus* m_{CO_2}) are plotted to evaluate the effect of pressure and temperature upon the process of SFE of grape seed oil. All extraction curves exhibit an initial linear period followed by a smooth asymptotic plateau (which represents 3–8% of the total oil extracted), tending to the same maximum, $\eta \cong 11.5\%$. This behaviour supports the broken + intact cells model of Sovová [25,26] which embodies a two-mechanism process, comprehending a rapid extraction from seed surface (i.e., oil convection from broken cells) followed by a diffusion-controlled period from inner intact cells.

The initial extraction rate increases with increasing pressure and/or decreasing temperature. Such findings must be due to the effect of P and T upon the oil solubility and mass transfer coefficients. In Table 1 the calculated CO_2 density, oil solubility (y_s) and convective mass transfer coefficient (k_f) are shown as function of P and T.

From Fig. 2 it is possible to observe the large impact of pressure on the extraction curves. At 313.15 K, the slopes of the first period of extraction at 180, 200 and 220 bar are 2.8, 3.9 and 5.6%/kg, respectively, and at 323.15 K they are 2.2 and 2.8%/kg for 180 and 200 bar. Attending to Table 1, these results may be attributed mainly to the solubility, since it affects directly the driving force to mass transfer: at 313.15 K, y_s increases up to 56% (from 180 to 220 bar), while k_f drops 14%, which means the y_s increment overlaps the negative variation of k_f; additionally, at 323.15 K y_s rises 42% while k_f diminishes 11% (from 180 to 200 bar). The oil solubility and the convective mass transfer coefficient have been estimated by the correlations of del Valle and Aguilera [27] and Tan et al. [28], respectively. The del Valle and Aguilera's correlation provide acceptable estimates for grape seed oil solubility in this work, as the concentration of esterified fatty acids is between 89.0 and 95.3%. Previous essays (e.g., Sovová et al. [29], Esquível and Bernardo-Gil [30]) have showed

Table 1
Calculated values of CO_2 density, oil solubility, and convective mass transfer coefficient for the experimental conditions studied.

P (bar)	T (K)	ρ^a (kg/m^3)	$y_s \times 10^{3\,b}$ (kg/kg)	$k_f \times 10^{6\,c}$ (m/s)
180	313.15	819.7	2.68	7.31
200		840.2	3.41	6.73
220		857.7	4.17	6.26
180	323.15	756.7	2.01	9.62
200		784.2	2.85	8.54

[a] Calculated with Bender's equation [19].
[b] Estimated with del Valle and Aguilera's correlation [27].
[c] Calculated with Tan et al.'s correlation [28]. The required binary diffusivity and CO_2 viscosity were calculated by the equations of Catchpole and King [34].

Table 2
Triacylglycerides total content (%, w/w) of oil samples obtained by Soxhlet and SFE (extracts and global oil).

Oil sample	Removal[a] (%)	P (bar)	T (K)	Average (%, w/w)	σ (%, w/w)
$E_1 + E_2$	13	200	313.15	86.7	2.1
E_4	55			92.4	2.6
E_7	100			89.8	1.8
E_1	14		323.15	88.2	1.0
E_4	60			96.6	4.5
E_7	96			91.9	1.8
Global oil[b]	100	180	313.15	92.7	2.5
			323.15	92.5	4.1
		200	313.15	90.8	1.2
			323.15	89.0	1.9
		220	313.15	95.3	2.6
Soxhlet			–	94.1	2.0
Commercial			–	97.3	0.3

[a] Percentage of extracted oil relative to maximum yield ($\eta \cong 11.5\%$).
[b] Oil obtained by joining all extracts together.

that free fatty acids may increase significantly the oil solubility, but such large deviations have been found for oils or oil fractions containing between 18 and 43% of free fatty acids.

With respect to the impact of temperature on the cumulative curves, Fig. 2 points out that it is more pronounced at higher pressure. In fact, at 180 bar the slopes are 2.8%/kg (at 313.15 K) and 2.2%/kg (at 323.15 K), whereas at 200 bar attain 3.9%/kg (at 313.15 K) and 2.9%/kg (at 323.15 K). These results emphasize once more the main role played by solubility in opposition to the weak effect of the mass transfer coefficient, because y_s decreases 25 and 16% whereas k_f increases 32 and 27%, respectively (see Table 1).

It is interesting to note that the first periods of extraction for 180 bar/313.15 K and 200 bar/323.15 K are practically coincident (the slopes are ca. 2.8 kg^{-1}), because the implied oil solubilities are comparable (2.68 and 2.85 kg/kg).

3.2. Oil composition along SFE

In this work, the grape seed oil content in triacylglycerides, and its fatty acids profile have been measured along the supercritical fluid extractions run at 200 bar/313.15 K and 200 bar/323.15 K. In Table 2, the total triacylglycerides content of three selected extracts of the previous curves ($E_1 + E_2$ or E_1, E_4 and E_7) shows that they are almost invariant along time (*Note*: at 313.15 K, the mass of extract E_1 was insufficient for the analysis, thus it has been joined to E_2).

In the whole, the triacylglycerides content of the global oil (i.e., all extracts mixed together) obtained at 180, 200 and 220 bar, and 313.15 and 323.15 K lies between 89.0 and 95.3%. It is worth noting the grape seed oil extracted by conventional Soxhlet produces similar results, namely an average composition of 94.1%, while a commercial oil analysed presented a value of 97.3%.

In Fig. 3 the fatty acids profiles of the $E_1 + E_2$ or E_1 (first extract) of both SFE curves (200 bar/313.15 K and 200 bar/323.15 K), Soxhlet extracted oil, and commercial oil are depicted. The major acids detected are palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and small amounts of linolenic (18:3). Other components may include free fatty acids, fat-soluble antioxidants and other fat-soluble compounds. As for the total triacylglycerides content cited above, no significant discrepancies were observed between the fatty acids composition of the extracts obtained by SFE at 313.15 and 323.15 K: palmitic acid, 5 and 8%; stearic acid, 4 and 6%; oleic acid, 14 and 17%; linoleic acid, 60 and 65%; linolenic acid, 0.3 and 0.4%; traces of palmitoleic acid (less than 0.1%). SFE does not change the high percentage of unsaturated fatty acids characteristic of grape seed oil which, in this case, totalizes 75–80%.

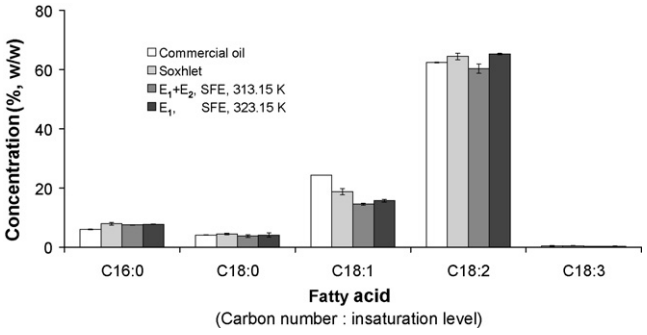

Fig. 3. Fatty acids profile of a commercial oil sample, Soxhlet extracted oil, and the individual extracts $E_1 + E_2$ and E_1 from 200 bar/313.15 K and 200 bar/323.15 K curves, respectively.

Furthermore, Fig. 3 shows these results compared to those obtained for the Soxhlet extracted oil, and for a commercial oil. Such findings are corroborated by Gómez et al. [6], who concluded that no significant differences of the fatty acids composition of oils extracted by *n*-hexane or with supercritical CO_2 at 350 bar and 313.15 K were observed. However, in our work the triacylglycerides recovered by Soxhlet and SFE are equivalent (94.1 and 89.0–95.3%, see Table 2), while Gómez et al. [6] reported very dissimilar values, as the free fatty acids content is only 3.4% for SFE and attained 33.8% by Soxhlet. In a different essay [31], the fatty acid analysis of grape seed oils of eight varieties obtained with petroleum ether and supercritical carbon dioxide gave results in agreement with the well accepted ranges published in specialized literature [32]. Moreover, experiments provided little dissimilarities between the oils extracted by both extraction methods.

3.3. Antioxidant capacity (AOC)

The antioxidant capacity of the oil obtained by SFE and Soxhlet has been measured and expressed in terms of tocopherol equivalents as explained in Section 2.6. This characterization has been also performed along two extraction curves, namely at 200 bar/313.15 K and 200 bar/323.15 K, in order to evaluate the effect of temperature on the AOC trend.

In Fig. 4 the antioxidant capacity of the final oil obtained by joining all extracts together is presented as function of the SFE operating conditions. The results show that the AOC increases with increasing pressure and/or temperature, although temperature imparts the strongest effect. For instance, at 313.15 K the equivalent tocopherol concentrations are 5.8, 6.2, and 7.4×10^{-4} M for 180, 200 and 220 bar, respectively. Nonetheless, at 180 bar the AOC jumps from 5.8 to 8.6×10^{-4} M, when temperature increases

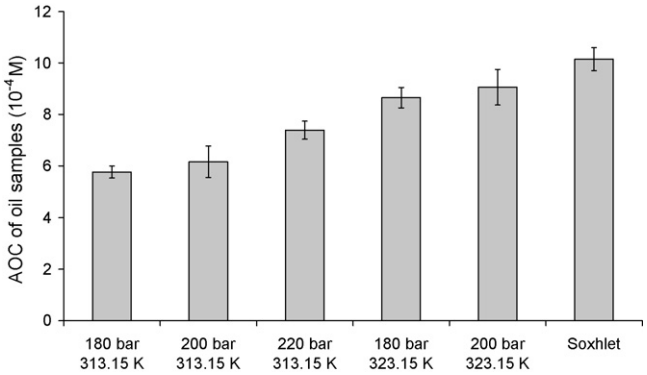

Fig. 4. Antioxidant capacity (AOC) of the extracted oil, expressed as concentration of an equivalent tocopherol solution.

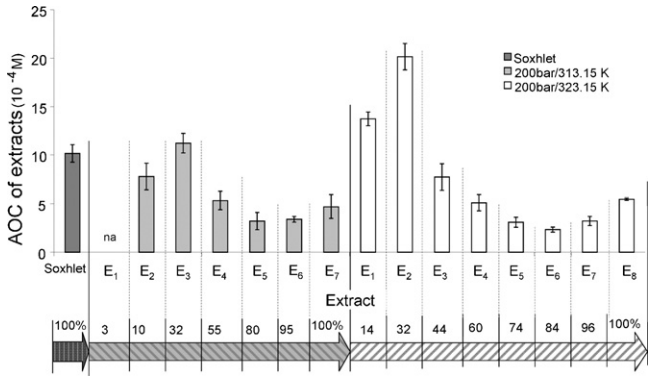

Fig. 5. Antioxidant capacity (AOC) of extracts along cumulative curves for 200 bar/313.15 K and 200 bar/323.15 K, expressed as concentration of an equivalent tocopherol solution; Soxhlet value is given for comparison. The labels over horizontal arrows are the percentages of extracted oil relative to maximum yield ($\eta \cong 11.5\%$). na: not measured.

from 313.15 to 323.15, and from 6.2 to 9.1×10^{-4} M at 200 bar. Such results may be interpreted by the influence that pressure and temperature exert on solubility, more precisely upon CO_2 density and vapour pressure of the interested antioxidant molecules. The increasing pressure increases solvent density, which enhances solubility. On the contrary, when temperature raises, the density decreases inherently, while solute vapour pressure increases instead. Nevertheless the last effect is more pronounced in this case, which implies larger solubilities and so higher antioxidant capacity.

Our results exhibit a similar behaviour to that reported by Bravi et al. [7]. In their work, the SFE of grape seed oil has been carried out at 250 bar and 313.15 and 353.15 K, and the α-tocopherol content determined by HPLC. Their observations demonstrated that the optimal extraction temperature is 353.15 K, where α-tocopherol concentration doubles for some experimental conditions. Nonetheless the AOC of the produced oil has not been specifically measured. It is worth noting that this property results from the combined and synergistic action of several compounds, notwithstanding the major role played by tocopherols.

None of the supercritical fluid extractions with CO_2 generated results as high as that obtained by conventional Soxhlet, i.e., 10.2×10^{-4} M or 0.049% (w/w) of tocopherol equivalents (see Fig. 4). This value is close to the limits of inclusion of antioxidants as food additives in most formulations, which correspond to levels up to 0.02–0.06% of the total fat weight [33]. The evolution of the AOC along the SFE of the grape seed oil assessed at 200 bar/313.15 K and 200 bar/323.15 K is shown in Fig. 5, where the AOC is represented for each extract. The bar for the oil obtained by Soxhlet is also graphed for comparison. It may be observed that the antioxidant activity is more pronounced in the first stages of the extraction, and at the end of each experiment, which denunciates that different classes of compounds with antioxidant capacity, or from distinct parts of the seed, may be recovered along extraction. Nonetheless, it is worth noting the sharp variation found at 200 bar/323.15 K at 32% of the extraction (i.e., from E_2 to E_3), a behaviour not so pronounced at 200 bar/313.15 K. The AOC of the first two fractions at 323.15 K almost doubles those at 313.15 K (7.8 and 11.2×10^{-4} M *versus* 13.7 and 20.2×10^{-4} M) whereas the remaining samples are not markedly different.

Taking these facts into account, and confronting the individual AOCs (Fig. 5) with those of the global oils obtained (Fig. 4), it is possible to conclude that the stages of the extraction period are responsible for the large difference found between the activities of the final oils: 6.2×10^{-4} M at 313.15 K and 9.1×10^{-4} M at 323.15 K.

Comparing the AOCs of the first extracts (i.e., until 32% of the complete extraction) with that from Soxhlet (see Fig. 5) one recognizes they are roughly similar at 200 bar/313.15 K (7.8 and 11.2×10^{-4} M *versus* 10.2×10^{-4} M), but visibly different at 200 bar/323.15 K (13.7 and 20.1×10^{-4} M *versus* 10.2×10^{-4} M). In this case, the first and second extracts alone overcome Soxhlet reference value by 34 and 98%, respectively. These results suggest a final remark on optimal operation policy for the supercritical fluid extraction of grape seed oil. It may be adopted with advantage an optimal temperature progression through the course of the extraction, to maximize the antioxidant activity of the natural oil and minimize the mass flow rate of supercritical CO_2. Accordingly, the temperature may be higher during the initial stages of extraction, to enhance antioxidant compounds removal, and reduced afterwards to decrease the CO_2 needed to recover the remaining oil.

4. Conclusions

In this essay, the SFE of grape seed oil using CO_2 has been carried out at different pressures and temperatures, namely 180, 200 and 220 bar, and 313.15 and 323.15 K. All extraction curves exhibit an initial linear extraction period followed by a smooth asymptotic plateau around $\eta \cong 11.5\%$. The extraction rate increases with increasing pressure and decreasing temperature, due to their effect upon the oil solubility essentially. The convective mass transfer coefficient plays a minor role under the scope of the experimental conditions run.

The triacylglycerides content of several extracts of the cumulative curves and of the final oils obtained have been measured, showing that it is roughly unaffected by the operating conditions adopted. The same occurred with the fatty acids profile. Nonetheless, the antioxidant capacity of grape seed oil, evaluated in this work in terms of tocopherol equivalents, depends on the extraction pressure and noticeably on the temperature. Results show that it increases with increasing pressure and increasing temperature, and that it is more pronounced on the oil extracted during the first stages of the process.

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References

[1] C.P. Passos, S. Yilmaz, C.M. Silva, M.A. Coimbra, Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail, Food Chemistry 115 (2009) 48–53.
[2] C. Crews, P. Hough, J. Godward, P. Brereton, M. Lees, S. Guiet, W. Winkelmann, Quantitation of the main constituents of some authentic grape-seed oils of different origin, Journal of Agricultural and Food Chemistry 54 (2006) 6261–6265.
[3] C.P. Passos, S.M. Cardoso, A.S. Barros, C.M. Silva, M.A. Coimbra, Application of FT-IR spectroscopy and O-PLS/PLS1 regression for estimation of Flavan-3-ols average degree of polymerization, Analytica Chimica Acta 661 (2010) 143–149.
[4] S. Bail, G. Stuebiger, S. Krist, H. Unterweger, G. Buchbauer, Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity, Food Chemistry 108 (2008) 1122–1132.
[5] A.C Rice, Solid-waste generation and by-product recovery potential from winery residues, American Journal of Enology and Viticulture 27 (1976) 21–26.
[6] A.M. Gómez, C.P. López, E.M. De la Ossa, Recovery of grape seed oil by liquid and supercritical carbon dioxide extraction: a comparison with conventional solvent extraction, Chemical Engineering Journal and The Biochemical Engineering Journal 61 (1996) 227–231.
[7] M. Bravi, F. Spinoglio, N. Verdone, M. Adami, A. Aliboni, A. D'Andrea, A. De Santis, D. Ferri, Improving the extraction of α-tocopherol-enriched oil from grape seeds by supercritical CO_2. Optimisation of the extraction conditions, Journal of Food Engineering 78 (2007) 488–493.
[8] T. Fuleki, J.M. Ricardo-da-Silva, Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario, Journal of Agricultural and Food Chemistry 45 (1997) 1156–1160.

[9] T. Escribano-Bailón, Y. Gutierrez-Fernández, J.C. Rivas-Gonzalo, C. Santos-Buelga, Characterization of procyanidins of *Vitis vinifera* variety Tinta del Pais grape seeds, Journal of Agricultural and Food Chemistry 40 (1992) 1794–1799.
[10] C. Saucier, M. Mirabel, F. Daviaud, A. Longieras, Y. Glories, Rapid fractionation of grape seed proanthocyanidins, Journal of Agricultural and Food Chemistry 49 (2001) 5732–5735.
[11] M. Pinelo, P.D. Fabbro, L. Manzocco, M.J. Nuñez, M.C. Nicoli, Optimization of continuous phenol extraction from *Vitis vinifera* byproducts, Food Chemistry 92 (2005) 109–117.
[12] Y. Yilmaz, R.T. Toledo, Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols, Journal of Food Composition and Analysis 19 (2006) 41–48.
[13] B. Díaz-Reinoso, A. Moure, H. Domínguez, J.C. Parajó, Supercritical CO_2 extraction and purification of compounds with antioxidant activity, Journal of Agricultural and Food Chemistry 54 (2006) 2441–2469.
[14] D.J. Huang, B.X. Ou, R.L. Prior, The chemistry behind antioxidant capacity assays, Journal of Agricultural and Food Chemistry 53 (2005) 1841–1856.
[15] I.M.G. Lopes, M.G. Bernardo-Gil, Characterisation of acorn oils extracted by hexane and by supercritical carbon dioxide, European Journal of Lipid Science and Technology 107 (2005) 12–19.
[16] M.C. Nicoli, M. Anese, M. Parpinel, Influence of processing on the antioxidant properties of fruit and vegetables, Trends in Food Science & Technology 10 (1999) 94–100.
[17] L. Fiori, Grape seed oil supercritical extraction kinetic and solubility data: critical approach and modeling, Journal of Supercritical Fluids 43 (2007) 43–54.
[18] E.J. Beckman, Supercritical and near-critical CO_2 in green chemical synthesis and processing, Journal of Supercritical Fluids 28 (2004) 121–191.
[19] G. Brunner, Gas Extraction—An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, 1st ed., Steinkopff Darmstadt, Frankfurt, 1994.
[20] 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, Journal of Supercritical Fluids 48 (2009) 225–229.
[21] J.C. Espín, C. Soler-Rivas, H.J. Wichers, Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2, 2-diphenyl-1-picrylhydrazyl radical, Journal of Agricultural and Food Chemistry 48 (2000) 648–656.
[22] N.P. Povh, M.O.M. Marques, M.A.A. Meireles, Supercritical CO_2 extraction of essential oil and oleoresin from chamomile (*Chamomilla recutita* L. Rauschert), The Journal of Supercritical Fluids 21 (2001) 245–256.
[23] S. Aued-Pimentel, J.H.G. Lago, M.H. Chaves, E.E. Kumagai, Evaluation of a methylation procedure to determine cyclopropenoids fatty acids from *Sterculia striata* St. Hil. Et Nauds seed oil, Journal of Chromatography A 1054 (2004) 235–239.
[24] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 4th ed., Person Education Limited, London, 2000.
[25] H. Sovová, Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation, Journal of Supercritical Fluids 33 (2005) 35–52.
[26] C.M. Silva, C.P. Passos, M.A. Coimbra, F.A. da Silva, Numerical simulation of supercritical extraction processes, Chemical Product and Process Modelling 4 (2009), Article 9.
[27] J.M. del Valle, J.M. Aguilera, An improved equation for predicting the solubility of vegetable oils in supercritical carbon dioxide, Industrial and Engineering Chemical Research 27 (1988) 1551–1553.
[28] C.S. Tan, S.K. Liang, D.C. Liou, Fluid solid mass-transfer in a supercritical fluid extractor, Chemical Engineering Journal and the Biochemical Engineering Journal 38 (1988) 17–22.
[29] H. Sovová, M. Zarevucka, M. Vacek, K. Stransky, Solubility of two vegetable oils in supercritical CO_2, Journal of Supercritical Fluids 20 (2001) 15–28.
[30] M.M. Esquível, G. Bernardo-Gil, Extraction of olive husk oil with compressed carbon dioxide, The Journal of Supercritical Fluids 6 (1993) 91–94.
[31] T.H.J. Beveridge, B. Girard, T. Kopp, J.C.G. Drover, Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects, Journal of Agricultural and Food Chemistry 53 (2005) 1799–1804.
[32] D. Firestone, Physical and Chemical Characteristics of Oils Fats and Waxes, American Oil Chemists' Society, Champaign, IL, 1997.
[33] R.D. O'Brien, Fats and Oils—Formulating and Processing for Applications, Technomic Publishing Company, Inc., Lancaster, 1998.
[34] O.J. Catchpole, M.B. King, Measurement and correlation of binary diffusion coefficients in near critical fluids, Journal of Industrial & Engineering Chemistry Research 33 (7) (1994) 1828–1837.