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Green extracts of grape seed oil - potential source of fatty acids and health benefits

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Supercritical CO_2 extraction of oil from grape seed samples obtained from a Portuguese industry without any previous treatment was carried out at temperatures from (313 to 333) K, pressures up to 40.0 MPa and different $scCO_2$ flow rates.

The qualitative analysis of the crude oil was carried out by NMR. The fatty acids were analyzed by GC-FID with reference to the parameters in Annex I to European Commission Regulation.

The results show similar content of triacylglycerols and diacylglycerols both in the *n*-hexane and $scCO_2$ extracts, but the latter have higher content of polyunsaturated fatty acids and lower content of saturated fatty acids, and hence are more beneficial for human health and wellbeing.

K**eywords:** Grape (*Vitis vinifera* L.) seed oil; supercritical CO_2 extraction; triacylglycerols; fatty acids.

INTRODUCTION

Seed biomass from *Vitis vinifera* L. contains typically (8–15) % (w/w) of oil which is rich in long chain polyunsaturated fatty acids (PUFAs) and antioxidants [1]. PUFAs are possibly less degradable than other fatty acids under particular conditions, and hence their presence in the extracts can increase the value of the oil obtained.

Development and implementation of sustainable processing concepts promotes reuse of residues of biomass. The biomass generated by the wine industry represents about 20-25 % of the total residues, and hence its recycling and reuse, grape seeds in particular, is of great importance since seed oil is beneficial for human health and wellbeing due to its high content of unsaturated fatty acids and antioxidant compounds [2, 3, 4].

Extraction of the pressed grape seeds with *n*-hexane is the current technique typically applied in an attempt to reuse the seeds biomass. However, a viable, green alternative extraction technique, applying supercritical CO_2 ($scCO_2$) as the solvent, can improve and reduce the environmental footprint. Supercritical fluids (especially $scCO_2$) possess a gas-like viscosity and diffusivity, and liquid-like density and solvating power [5, 6, 7], and have been applied and accepted as future industrial solvents, mainly in the field of thermolabile high value-added products. Supercritical extraction (SCE) of oil from grape seeds from different cultivars as well as SCE of different high-added value substances from grape seed oil have been studied and reported by other research groups, the following references [4], [8, 9, 10, 11] are just few examples of the most recent contributions in the respective field. However, data about the yield and characterization of grape seed oil obtained by SCE of industrial, without any previous treatment, samples are scarce. Hence, further investigation that will provide new experimental data, analyses results and determine appropriate operating conditions will contribute to the knowledge of how to improve the quality of the valuable green extracts and products obtained from grape seed biomass.

In view of the above, the aims of our work are to: *i*. Obtain oil extracts from grape seeds, supplied by a Portuguese industry, applying SCE and *n*-hexane extraction; *ii*. Compare the influence of the extraction method on the yield, and on the lipids and fatty acids composition of the extracts, with the view to determine which is more beneficial for human health.

MATERIALS AND METHODS

Grape seeds from the center of Portugal, separated and milled in advance, were provided by a Portuguese wine industry. The biomass was drying for a period of 48 h at 343 K, which resulted in a decrease of the mass within (9.0±0.6) %. Thermogravimetric process in an electronic balance was used to determinate moisture content in a (Kern MRS 120-3), at a temperature of 378 K. The result of a triplicate analysis was (2.43±0.21) %.

Supercritical CO_2 and organic solvent extraction

Supercritical CO_2 extraction was performed in a laboratory apparatus, shown in Fig. 1, equipped with a 50 cm^3 internal volume vessel, built from AISI 316 stainless steel tubing (32 cm long and an internal

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diameter of 1.41 cm). Liquid CO_2 from the cylinder (G) is compressed (C) to the required pressure, then passes via the heater system and after that through the extraction vessel (E), in which CO_2 flows through the matrix sample before expansion in the micrometer valve (MM).

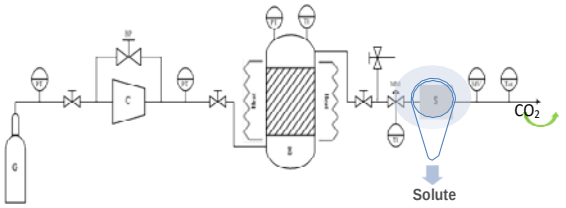

Fig. 1. Diagram of the supercritical fluid extraction apparatus. G, CO_2; C, compressor; E, extractor; S, separator; BP, back-pressure regulator; MM, micrometer valve; MV, flow meter; Tot, totalizer; TI, temperature indicator, PT, pressure indicator.

In our experiments the extracts were collected in a U tube (S), at atmospheric pressure and at a temperature controlled with a refrigerated bath. The amount of extract obtained was assessed gravimetrically with an uncertainty of ±0.1 mg. The total volume of CO_2 was determined with a mass flow meter and a totalizer from Alicat Scientific (USA), model M-5 SLPM-D (MV and Tot) [12, 13, 14]. CO_2 (purity 99.99 %) was supplied by GASIN - Air Products, Portugal.

The SCE was carried out using samples of 17 g of the grape seeds with particle size of (0.62±0.04) mm. Conditions of extraction were: CO2 flow rates of 0.11 kg/h, pressures up to 40 MPa, and temperatures up to 333 K. The conditions were controlled during all experiments, within (130-230) min time span, until no longer any significant mass of oil was recovered, which was considered as an indication that the extraction process was completed.

The Soxhlet extractions of the grape seeds to isolate the oil, were carried out with 250 mL of *n*-hexane (Sigma Aldrich) and 20 g of seeds samples, for 4 h at the solvent boiling point. The extract was filtered and the solvent removed by reduced pressure evaporation (rotavapor) to constant weight. The extraction yield was 12.28±0.35 % (w/w). The oil extract was kept at 253 K until analyzed.

Analysis of the crude oil extracts by ^1H NMR

The ^{1}H-NMR spectra of the crude oil extracts were obtained on a Bruker Avance 400 MHz NMR spectrometer (Bruker Inc., Bremen, Germany), with a 5 mm PABBO BB-1H probe using standard Bruker routines (90° proton pulse length of 11.8 µs and a delay time between acquisitions of 30 s). All spectra were taken at 298 K in $CDCl_3$ (500 µL, 75–100 mM grape seed oil) and the residual signal of the solvent was used as the internal reference. Chemical shifts (δ) were assigned based on previous reports [4], [15, 16].

Quantitative analysis of fatty acids (FAs)

Transesterification was carried out in a methanol solution of KOH (2M), following the necessary procedures as established in the Annex I to Commission Regulation (EEC) No 2568/91(1), CELEX_01991R2568 published 04.12.2016, with the necessary adaptations.

Gas chromatography of the fatty acid methyl esters (FAMEs) was performed using a fused-silica capillary column SP-2380, 60 m length, 0.25 mm of internal diameter, 0.20 µm film thickness, with helium as the carrier gas at a constant flow rate of 1.0 mL/ min.

The oven temperature in the GC was 438 K for 25 min; programmed heating from (438 to 483) K at 5 K/min and subsequent holding at 483 K for 10 min. The temperatures of the injector and detector were kept constant at (523 and 553) K, respectively. FAMEs were identified by comparing their retention times with those of a reference standard solution (supplied by Sigma-Aldrich) at the same condition.

RESULTS AND DISCUSSION

Supercritical fluid vs n-hexane extraction – oil yield and time

An evaluation of the effect of SCE operating parameters - pressure and temperature – on the extraction yields, as well as a comparison with the *n*-hexane extraction yield, can be deduced from Table 1.

Table 1. SCE times and oil yields, as a function of the operating conditions, at $scCO_2$ flow rate F = 0.11 kg·h^{-1}, as compared to *n*-hexane extraction results.

Extraction Method		Oil Yield (%)	Time (min)
	Hexane	12.28±0.35[a]	240
SCE conditions: *p*(MPa)/*T* (K)	20/313	7.20±0.50[b]	227
	30/313	11.96±0.60[a]	191
	40/313	12.07±0.55[a]	148
	30/333	12.17±0.38[a]	214
	40/333	12.83±0.56[a]	134

[a,b] In column two, the values with different letters are significantly different (p≤0.05), according to Tukey HSD test

One-way ANOVA with post-hoc Tukey HSD calculator was performed to determine differences between oil yields obtained by *n*-hexane and by SCE at the operating conditions of the experiment.

The maximum oil yields achieved by the SCE were in the range (12.0-12.8) % and were attained at the extraction conditions applied, the lower pressure of 20.0 MPa being an exception. The *n*-hexane extraction oil yield was 12.3 %. Moreover, the SCE times to obtain the maximum yields, compared with *n*-hexane extraction, were shorter - around 140 min for the higher pressure (40 MPa) and 200 min – for the case when the pressure was 30 MPa (Table 1).

The experimental SCE yields obtained are in agreement with the results of other authors [4], [9], [17, 18]. Yet, slight differences can be found in the literature [18, 19] regarding the overall evolution of the extraction of the oil, which can be attributed to a number of factors such as the origin of the vegetable matrices, pretreatment at industrial scale, particle size of the plant material, and the moisture content.

Quantitative analysis of the crude oil extracts

The crude grape seeds oil extracts were quantitatively analyzed by ^{1}H NMR. Fig. 2 shows the ^{1}H NMR spectrum of the grape seeds oil obtained by $scCO_2$ extraction as an example, and the relevant NMR signals used for determination of the chemical composition. The results of the ^{1}H-NMR quantitative analyses are shown on Fig. 3a, 3b and Fig. 4, which display a comparison of the lipids composition of the grape seed oils obtained by *n*-hexane and $scCO_2$ extraction, and of the fatty acids groups in the lipids, respectively.

The presence of 1,2-DAGs in the oil samples was determined by the signal at δ_H 3.72 ppm attributed to the glyceryl methylene protons at sn-3 position, as well as the oxidized lipids (linolenic hydroperoxides), by the characteristic olefinic protons of the conjugated diene system in the region 6.60-5.70 ppm. Fig. 3a shows that the lipid composition of both the SCE and *n*-hexane extracts is largely dominated by triacylglycerols (TAGs, 95-98 %). Other compounds like of 1,2-diacylglycerols (1.6–3.5 %) and oxidized lipids (0.4–1.8 %) represent only a minor contribution to the overall composition of the oil extracts as displayed on Fig. 3b. It should be noted, that the results obtained by us are dependent on the extraction conditions and on the vegetable matrix origin [4, 18], and are in a good agreement with literature data.

The fatty acids content of the grape seeds oils can also be evaluated by the relative integration of the ^{1}H-NMR signals attributed to the hydrocarbon chains with different number of unsaturations. The signal at δ_H 2.30 ppm (**a**) attributed to the methylene group adjacent to the carbonyl group and present in all the fatty ester derivatives was chosen to determine and normalize the integrations of the other NMR signals. The relative integration determines the distribution between the monounsaturated (MUFA) (signal **b**) and the diunsaturated (DUFA) (signal **c**) acyl chains on the glycerol backbone. The abundance of the saturated (SFA) chains is obtained as the difference between the total fatty acids (FA) and all the unsaturated (MUFA + DUFA) chains.

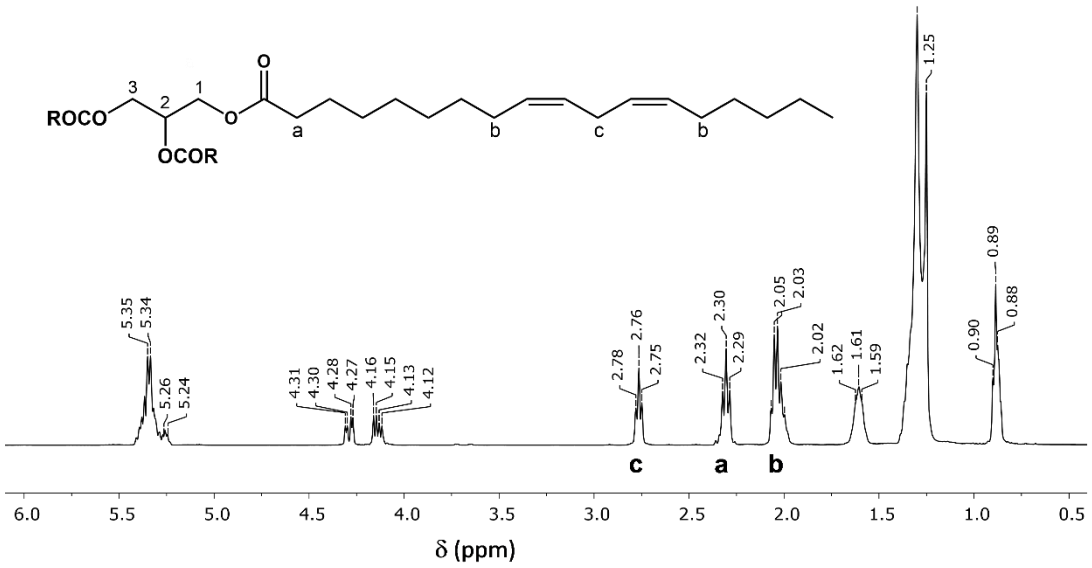

Fig. 2. ^{1}H-NMR spectrum of grape seed oil obtained by $scCO_2$ in $CDCl_3$ showing the attribution of the signals to specific protons in the linoleic acyl chain.

Fig. 3a **Fig. 3b**

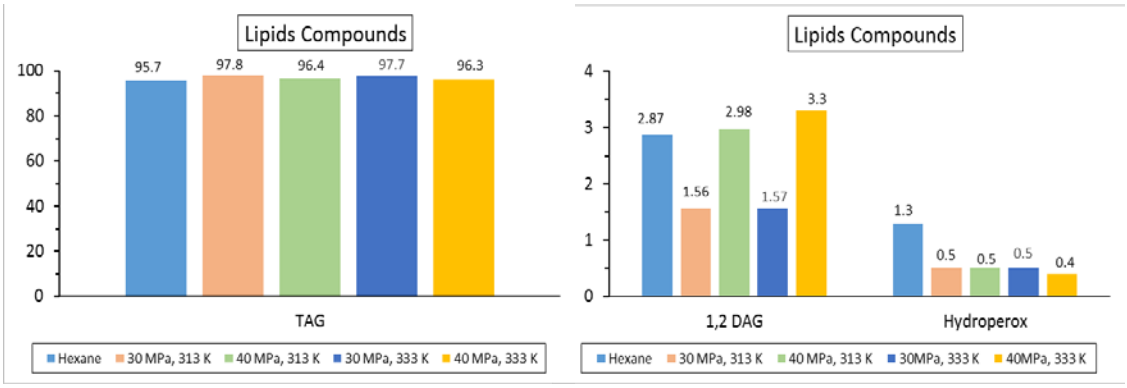

Fig. 3. Lipids composition of grape seed oils obtained by hexane and $scCO_2$ extraction at a flow rate of $scCO_2$ F = 0.11 $kg \cdot h^{-1}$, as established by ^{1}H-NMR quantitative analysis. All values represent % molar fractions.

Table 2. Fatty acid composition (% of total fatty acids) from FAME GC-FID analysis of grape seed oils obtained by hexane and $scCO_2$ extraction at $scCO_2$ flow rate F = 0.11 $kg \cdot h^{-1}$.

Fatty acid	Hexane	$scCO_2$ conditions: p(MPa)/T(K)			
		30/313	40/313	30/333	40/333
C14:0 - myristic	0.06	0.05	0.05	0.05	0.06
C16:0 - palmitic	8.13	7.53	7.48	7.59	7.38
C16:1 - palmitoleic	0.12	0.12	0.11	0.12	0.11
C17:0 - margaric	0.06	0.06	0.06	0.06	0.06
C18:0 - stearic	5.61	4.91	5.02	4.85	5.04
C18:1 - oleic	20.64	19.22	19.24	19.18	19.27
C18:2 - linoleic	64.52	67.30	67.17	67.37	67.23
C18:3 - linolenic	0.30	0.34	0.34	0.32	0.33
C20:0 – arachidic	0.23	0.18	0.2	0.17	0.21
C20:1 – gadoleic	0.19	0.17	0.18	0.15	0.17
C22:0 – behenic	0.04	0.04	0.04	0.05	0.04
C24:0 - lignoceric	0.07	0.04	0.04	0.04	0.04

Uncertainties in the values of composition (x) are: 0.003<x< 0.1 ±0.03; 0.1<x<1 ±0.05; 1<x<10 ±0.13 and ≥10±0.52

The unsaturation index (UI), defined as UI = (2×DUFA % molar fraction + MUFA % molar fraction)/100 is an important parameter which defines the ratio of these compounds. All supercritical oil extracts obtained contain higher percentages of DUFAs and similar MUFAs, when compared to the hexane extract, and hence possess higher values of UI.

Quantitative analysis of fatty acids (FAs)

Table 2 shows the fatty acid composition (% of total fatty acids) analysis of grape seed oils obtained by *n*-hexane and $scCO_2$ extraction. These results are in a good agreement (see Fig. 4) with those obtained by NMR analysis, with the exception of the hexane extract for which a minor disagreement with regard to the DUFA represented by C18:2 – linoleic can be found. The major fatty acids are the C18:2 – linoleic (64.5-67.47 %); C18:1 – oleic (19.18-20.64 %); C16:0 – palmitic (7.38-8.22 %) and C18:0 – stearic (4.33-5.61 %). The results in Table 2 confirm that DUFAs are the principal fatty acids present in the grape seeds oils, followed by the MUFAs and SFAs.

As suggested by Garavaglia *et al*. [20], high content of MUFAs in foods and diets is very important, because, for example, MUFAs may help lower the risk of heart disease by lowering the total and low-density lipoprotein (LDL) cholesterol levels while maintaining high-density lipoprotein (HDL) cholesterol level.

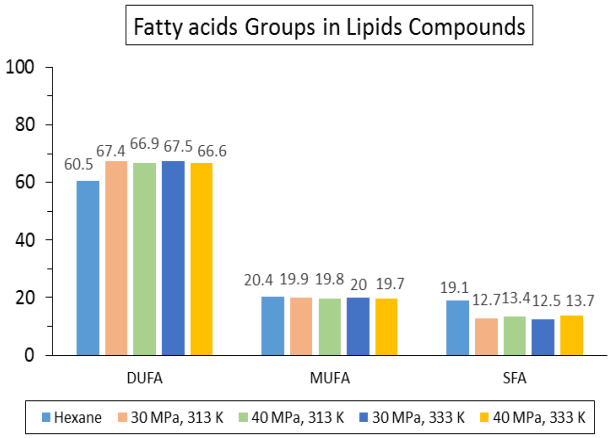

Fig. 4. Fatty acids groups in lipids composition of grape seed oils obtained by hexane and $scCO_2$ extraction at a flow rate of $scCO_2$ F = 0.11 $kg \cdot h^{-1}$. All values represent % molar fractions.

CONCLUSIONS

The influence of SCE operating parameters – temperature and pressure - on the yield and the fatty acid profile of the oil extracted from industrial grape seeds biomass were analyzed in detail and reported.

The highest oil yields achieved by the SCE were in the range 12.0-12.7 %, as compared to 12.3 % obtained by a conventional *n*-hexane extraction. However, in the former case, not only a free solvent extract can be obtained, but also the extraction times are lower. The main fatty acids present in the $scCO_2$ oil extracts are linoleic and oleic acids, with an average percentage of (67 and 20) %, respectively.

Taking into consideration the more favorable unsaturation index (UI) of, and the higher linoleic acid content in, the $scCO_2$ oil extracts, as compared to those obtained by the conventional *n*-hexane extraction, it can be concluded that SCE is the appropriate environmentally benign process to achieve a high quality grape seed oil extracts that can be used as an excellent food and diet supplement.

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МАСЛО ОТ ГРОЗДОВИ СЕМКИ ПОЛУЧЕНО СЪС СВРЪХКРИТИЧНА ЕКСТРАКЦИЯ - ИЗТОЧНИК НА МАСТНИ КИСЕЛИНИ И ПОЛЗИ ЗА ЗДРАВЕТО

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(Резюме)

В настоящата работа са проведени екстракции със свръхкритичен въглероден диоксид (СКФ) на масло от гроздови семки. Пробите са получени директно от португалска индустрия без предварителна обработка. Експериментите са проведени при температури от (313 до 333) K, налягания до 40.0 MPa и различни скорости на потока на СКФ. Качественият анализ на суровото масло бе извършен чрез NMR. Мастните киселини се анализираха с GC-FID по отношение на параметрите в приложение I към Регламента на Европейската комисия. Получените резултатите показаха, че съдържанието на триацилглицероли и диацилглицероли в екстрактите получени с n-хексан е подобно на това в екстрактите получени със СКФ. Последните, обаче, имат по-високо съдържание на полиненаситени мастни киселини и по-ниско съдържание на наситени мастни киселини и следователно са по-полезни за човешкото здраве и благополучие.