

Extraction of Grape Seed Oil Using Compressed Carbon Dioxide and Propane: Extraction Yields and Characterization of Free Glycerol Compounds

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The main objective of this work was to compare the extraction of grape seed oil with compressed carbon dioxide and propane on the extraction yields and chemical characteristics of free glycerol compounds. The experiments were performed in a laboratory scale unit in the temperature range of 30 to 60 °C and pressures from 60 to 254 bar. The results showed that propane is a more suitable solvent for grape seed oil extraction than carbon dioxide, as higher extraction yields and a very fast kinetic of extraction were achieved with this solvent. In relation to compressed carbon dioxide extractions, both temperature and density presented a very pronounced and positive effect on the extraction yield. The oils extracted were analyzed qualitatively and quantitatively with regard to the free glycerol compounds, mainly fatty acids, ethyl, and methyl esters. The results showed that these compounds are present in low concentration in vegetable oil (<3%) and that, in general, samples extracted with propane present a smaller amount of peaks of free glycerol compounds in the oil than samples extracted with carbon dioxide.

KEYWORDS: SFE; CO₂ extraction; grape seed oil; propane extraction

INTRODUCTION

The extraction with supercritical fluid (SFE) is described in the literature as an alternative to classic extraction methods (1–6). Carbon dioxide is the most commonly used solvent in SFE, mainly due to its physical and chemical properties, such as low critical pressure (72.8 atm) and temperature (31 °C) and chemical inertness (3, 7, 8). Supercritical CO₂ has good solvent properties for extraction of nonpolar compounds such as hydrocarbons. However, its large quadrupole moment also enables it to dissolve some moderately polar compounds such as alcohols, ethers, aldehydes, and ketones (5). Carbon dioxide has advantages compared to liquid solvents due to its adjustable solvent power and properties, ranging from gas to liquid. It should also be considered that extracts can be obtained without traces of solvent in the end of the process. In this sense, at selected temperature and pressure conditions, pressurized CO₂ is a natural solvent for the oil extraction process.

Besides CO₂, other compressed solvents can be used, such as ethane, propane, propylene, and ethylene, among others. On the other hand, many scientists attest that these solvents should not be used because of high cost, danger, toxicity, and inflammability (9, 10). Propane does not present many of the qualities of CO₂, but in some situations, it could be a better solvent. Propane is relatively inexpensive and does not leave a toxic residue. Besides, the pressures involved in oil extraction using propane are at least an order of magnitude (hundreds of psi compared with thousands of psi) lower than those in SC-CO₂ extraction of vegetable oils (11). Its low critical temperature and similarity to organic compounds, if compared to CO₂, renders propane economically advantageous in some separation processes (12).

Some studies compare the solubility of compounds in SFE-CO₂ and subcritical propane extraction for paprika analysis (13), coriander seed (10), and hiprose fruit (14). The results showed that better yields for capsaicinoids, carotenoids, and tocopherols are obtained in the extraction with propane, while similarity results were achieved for fatty acid compounds (10, 14). The phase behavior studies of olive, castor, and soybean oils at high pressure also demonstrate that the miscibility pressure using

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Table 1. Compounds Used for Quantitative Analysis of the Grape Seed Oil

peak	name	ions	peak	name	ions
1	methyl laurate	74	12	methyl stearate	74
2	lauric acid ^a	73	13	ethyl linoleate	88
3	methyl myristate	74	14	ethyl oleate	88
4	ethyl myristate	88	15	ethyl stearate	88
5	myristic acid ^a	73	16	linoleic acid ^a	73
6	methyl palmitoleate	74	17	oleic acid ^a	73
7	methyl palmitate	74	18	stearic acid ^a	73
8	ethyl palmitate	88	19	methyl araquidate	74
9	palmitic acid ^a	73	20	ethyl araquidate	88
10	methyl linoleate	74	21	methyl lignocerate	74
11	methyl oleate	74			

^a Identified as a TMS derivative.**Table 2.** Process Conditions and Yields Obtained from the Extraction of Grape Seed Using Pressurized Carbon Dioxide and Propane as Solvents

experimental condition	solvent	pressure [bar]	temperature [°C]	solvent density [g/cm ³]	reduced density	yield
1	CO ₂	60.0	45	0.389	0.83	0.06
2	CO ₂	108.0	45	0.516	1.10	0.69
3	CO ₂	118.4	45	0.656	1.40	1.76
4	CO ₂	181.6	45	0.797	1.70	6.38
5	CO ₂	250.0	45	0.867	1.85	7.96
6	CO ₂	110.0	30	0.797	1.70	3.52
7	CO ₂	254.0	60	0.797	1.70	7.93
8	propane	100.0	30	0.510	2.35	10.43
9	propane	100.0	45	0.489	2.25	10.82

propane is much lower than that using carbon dioxide as solvent (15–17).

Few works have been reported for the extraction of grape seed using SC-CO₂ alone (18) or with modifiers (19–22). These works are focused on the supercritical extraction with CO₂ on yields and composition of the grape seed oil. On the other hand, there is a lack of information regarding the comparison of solvent extraction of grape seed oil, mainly regarding compressed hydrocarbon solvents that are more efficient solvents for vegetable oils than carbon dioxide. In this sense, the present study was undertaken to investigate the effects of SFE-CO₂ and

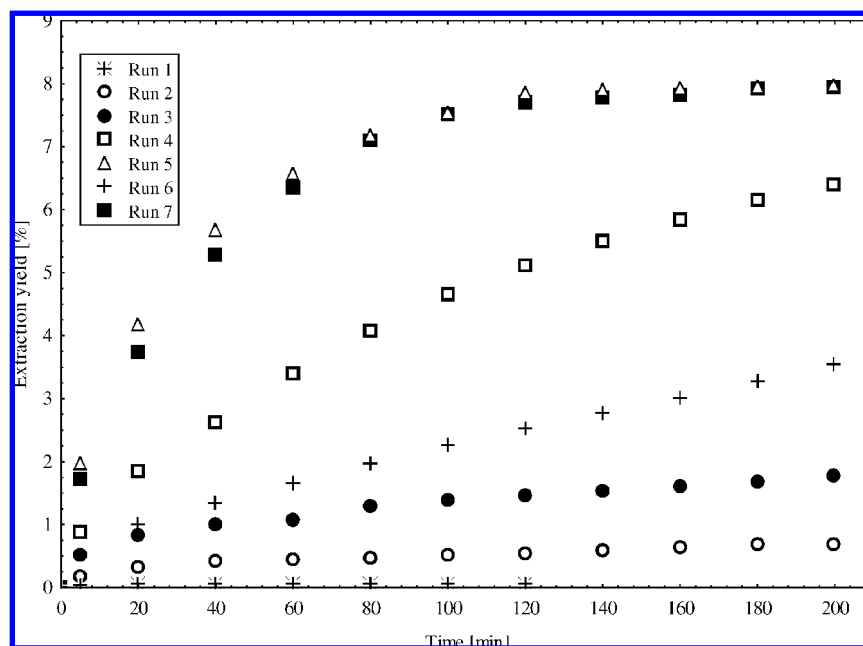
subcritical propane on the extraction yields of grape seed oil and also on the chemical characteristics of the free glycerol compounds presented in the extracted oils.

MATERIALS AND METHODS

Materials. Grape seed samples (mixture of Isabel and Herbemont species) were collected after the process of wine fermentation at Aurora LTDA, a local industry of wine. The samples were washed in distilled water, dried, milled, and stored under refrigeration and protected from light until the extraction analysis.

All solvents used in this study were of analytical grade. The standard esters, methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, and methyl arachidate, ethyl palmitate, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl linolenate, and ethyl arachidate (all $\geq 99\%$), and fatty acids (palmitic, stearic, oleic, linoleic, arachidic, and lignoceric) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Fluka (Buchs, Switzerland). The derivatizing reagent bis(trimethylsilyl)trifluoroacetamide) was utilized for GC analysis of volatile compounds. Carbon dioxide (CO₂) and propane were technical grade and obtained from White Martins (Brazil).

Extraction Apparatus and Procedure. The experiments were performed in “home-made” equipment that was described already extensively in other works of our research group, related to the extraction with pressurized CO₂ of plants and essential oil (23–27). For this reason, only a brief description is presented here. The experimental unity consists basically of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 100 cm³ jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of 0.12 bar, a collector vessel with a glass tube, and a cold trap. Amounts of around 10 g of dried of finely comminuted seeds (average particle size of 0.14 mm) were charged into the extraction vessel. The solvent was pumped at a constant flow rate of 2 mL/min into the bed, which was supported by two 300 mesh wire disks at both ends and was kept in contact with the herbaceous matrix for at least 30 min to allow system stabilization. Afterward, the extract was collected by opening the micrometering valve, and the solvent mass flow was accounted for by the pump recordings. After that, the mass of the extracted oil was weighed, and the glass tube was reconnected to the equipment. This procedure was performed until no significant mass was extracted or, as in some cases, the extraction period exceeded a pre-established limit. For carbon dioxide extractions, the collection of oil was established by 20 per 20 min, while in the extractions using

**Figure 1.** Kinetic of grape seed oil extraction using pressurized carbon dioxide. Experimental conditions are labeled according to Table 2.

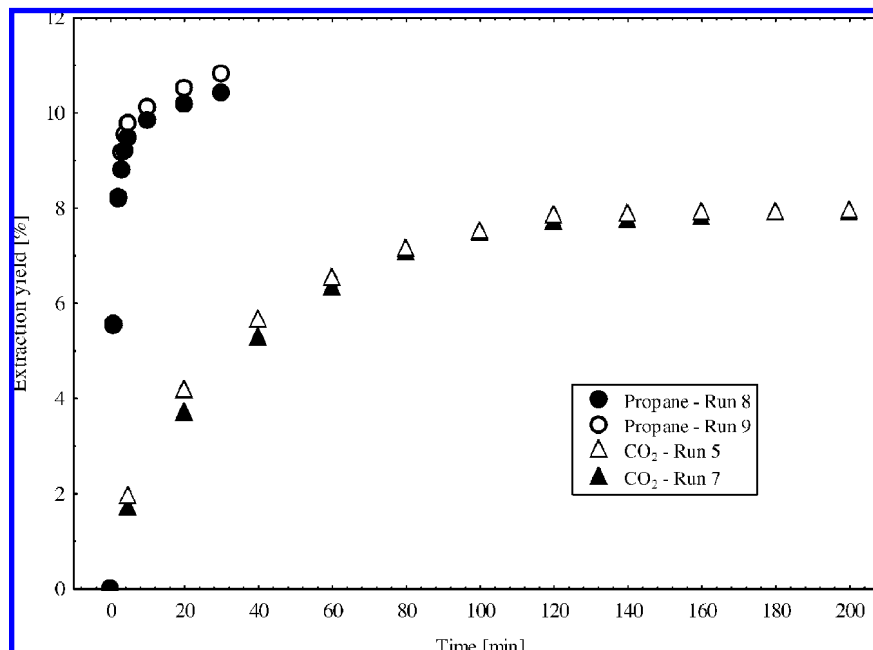


Figure 2. Comparison of the extraction kinetic of grape seed oil using carbon dioxide and propane. Runs are labeled according **Table 2**.

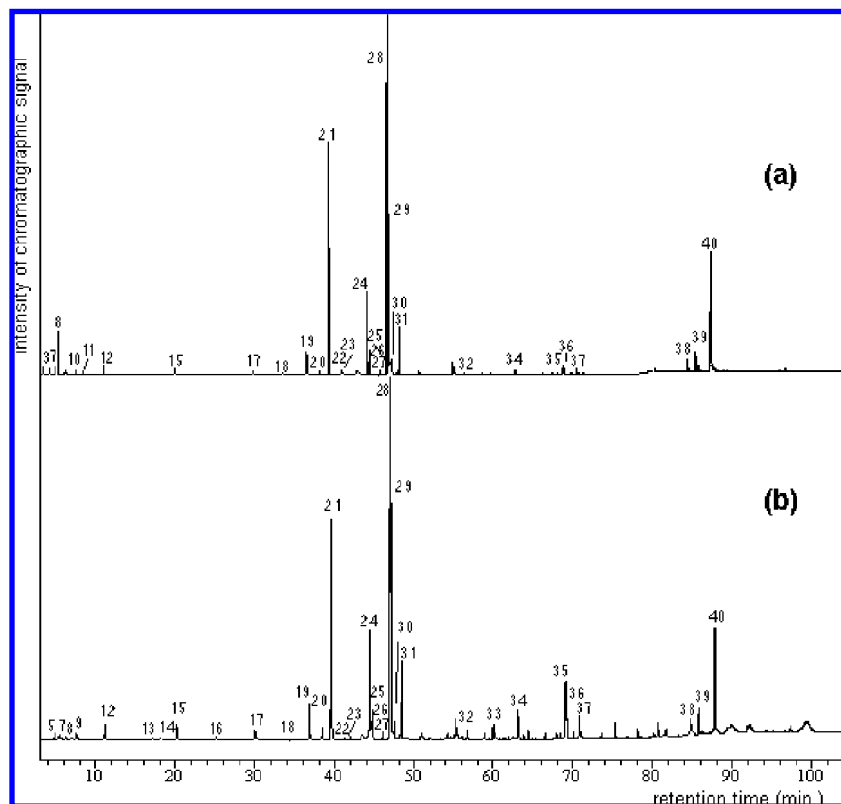


Figure 3. Typical total ion chromatogram (TIC) of grape seed oil extracted with (a) CO₂ and (b) propane.

propane as solvent the collection was accomplished by 1 per 1 min up to 5 min and by 5 per 5 min up to 30 min.

The experiments were accomplished isothermally at constant pressure. A whole experimental run lasted in general 8 h, including all steps involved: sample weighing, temperature stabilization (baths, extractor), extraction, and depressurization. The experimental range investigated was 30 to 60 °C in temperature and from 60 to 254 bar in pressure. Duplicate runs were performed for all conditions, leading to an overall relative standard deviation of the yields of about 0.2%.

The experimental conditions were selected to evaluate the effect of solvent density and temperature on the extraction yield. In this sense, temperature was fixed at 45 °C and extractions were performed using

supercritical carbon dioxide at distinct reduced densities (solvent density/critical solvent density) (runs 1 to 5). The effect of temperature was evaluated at 30, 45, and 60 °C at fixed reduced density of 1.70.

Analysis of the Free Glycerol Compounds. The fatty acids present in the grape seed oil were converted with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in pyridine to volatile trimethylsilyl ester derivatives. Around 20 mg of the oil sample was dissolved in dichloromethane and derivatized with 20 μ L of BSTFA. The solution was mixed and stood for 30 min at room temperature.

The quantification of fatty acids, ethyl, and methyl esters was performed using a Shimadzu 17A gas chromatograph coupled with a

Table 3. Identification of Compounds in the Grape Seed Oil Extracts (Carbon Dioxide and Propane)

no.	comps	name	extractions						
			CO ₂			propane			
			run 2	run 3	run 4	run 5	run 7	run 8	run 9
1	C ₉ H ₂₀ O ₂ Si	caproic acid ^a							
2	C ₁₀ H ₂₀ O ₂	ethyl caprilate							
3	C ₁₁ H ₁₈ O ₂ Si	ethanol phenyl ^a	X			X	X		X
4	C ₁₂ H ₂₄ O ₂	ethyl caprate							
5	C ₉ H ₁₈ O ₄ Si ₂	ethyl succinate	X	X		X			
6	C ₁₀ H ₂₂ O ₄ Si ₂	succinic acid ^a	X						
7	C ₁₁ H ₂₄ O ₂ Si	caprylic acid ^a	X	X	X	X			X
8	C ₁₂ H ₃₂ O ₃ Si ₃	glycerol ^a	X	X		X	X		X
9	C ₁₀ H ₁₆ O	decadienal	X	X	X	X			
10	C ₁₂ H ₂₆ O ₂ Si	pelargonic acid ^a	X		X		X	X	X
11	C ₁₂ H ₂₄ O ₂	ethyl caprate			X	X			X
12	C ₁₃ H ₂₈ O ₂ Si	capric acid ^a	X	X	X	X	X	X	X
13	C ₁₄ H ₂₈ O ₂	ethyl laurate		X		X	X		
14	C ₁₀ H ₂₀ O ₄ Si ₂	fumaric acid ^a		X		X			
15	C ₁₅ H ₃₂ O ₂ Si	lauric acid ^a	X	X	X	X	X	X	X
16	C ₁₆ H ₃₂ O ₂	ethyl mirystate		X					
17	C ₁₇ H ₃₆ O ₂ Si	myristic acid ^a	X	X		X	X		X
18	C ₁₇ H ₃₄ O ₂	methyl palmitate	X		X		X		X
19	C ₁₈ H ₃₆ O ₂	ethyl palmitate	X	X	X	X	X	X	X
20	C ₁₉ H ₃₈ O ₂ Si	palmitoleic acid ^a	X	X	X	X	X		X
21	C ₁₉ H ₄₀ O ₂ Si	palmitic acid ^a	X	X	X	X	X	X	X
22	C ₁₉ H ₃₄ O ₂	methyl linolenate	X	X					X
23	C ₁₉ H ₃₆ O ₂	methyl oleate	X	X					X
24	C ₂₀ H ₃₆ O ₂	ethyl linoleate	X	X	X	X	X	X	X
25	C ₂₀ H ₃₈ O ₂	ethyl oleate	X	X	X	X	X	X	X
26	C ₂₀ H ₃₄ O ₂	ethyl linolenate	X	X	X	X			X
27	C ₂₀ H ₄₀ O ₂	ethyl stearate	X	X	X	X	X	X	X
28	C ₂₁ H ₄₀ O ₂ Si	linoleic acid ^a	X	X	X	X	X	X	X
29	C ₂₁ H ₄₂ O ₂ Si	oleic acid ^a	X	X	X	X	X	X	X
30	C ₂₁ H ₃₈ O ₂ Si	linolenic acid ^a	X	X	X	X	X	X	X
31	C ₂₁ H ₄₄ O ₂ Si	stearic acid ^a	X	X	X	X	X	X	X
32	C ₂₃ H ₄₃ O ₂ Si	araquidic acid ^a	X	X			X		X
33	C ₂₅ H ₅₂ O ₂ Si	lignoceric acid ^a	X	X		X	X		
34	C ₂₅ H ₅₄ O ₄ Si ₂	monopalmitin ^a	X	X		X	X		X
35	C ₂₇ H ₅₆ O ₄ Si ₂	monolinolein ^a	X	X		X	X		X
36	C ₂₇ H ₅₈ O ₄ Si ₂	monoolein ^a	X	X		X	X		X
37	C ₃₀ H ₅₀	squalene	X	X	X	X	X	X	X
38	C ₂₈ H ₄₈ O	brassicasterol			X	X	X	X	X
39	C ₂₉ H ₄₈ O	stigmastadienol			X			X	X
40	C ₂₉ H ₅₀ O	clionasterol			X			X	X
41	C ₃₁ H ₅₆ O ₂ Si	campesterol ^a	X	X		X			
42	C ₃₂ H ₅₆ O ₂ Si	stigmasterol ^a	X	X		X	X		
43	C ₃₂ H ₅₈ O ₂ Si	sitosterol ^a	X	X		X	X		

^a Identified as a TMS derivative.

mass detector QP 5050A, fitted with a capillary column (60 m × 0.25 mm i.d.) that was packed with 5% phenyl silicon (0.25 μm of phase thickness). The temperature column was initially maintained at 120 °C for 5 min and then raised to 280 °C at a rate of 2 °C/min, staying at this temperature for 20 min. The total run time was 105 min. The carrier gas was set at 40 psi with a flow rate of 1 mL/min. The analyses were performed with the injector and detector at 280 and 300 °C, respectively, and the injection volume was 1.0 μL in the 1:10 split mode. Mass spectra were scanned from *m/z* 55 to 650 Da, at a rate of 1.5 scans/s. The electron impact ionization energy was 70 eV. The identification of compounds presented in the grape seed oil was conducted by comparison of the spectrum data with those presented in the Willey library.

For the quantification of fatty acids, ethyl, and methyl esters presented in **Table 1**, the GC oven was operated at a starting temperature of 200 °C, held for 8 min, and then heated at 3 °C/min to 235 °C, and after heated at 15 °C/min to 280 °C and held at 280 °C for 15 min.

RESULTS AND DISCUSSION

Extraction Yield. **Table 2** presents the experimental conditions and extraction yields obtained from the extraction of grape

seed oil using pressurized carbon dioxide and propane as solvents. Extraction yield was defined in this work as 100 times the mass of oil extracted by the mass of grape seed raw material after a certain period of extraction. From **Table 2**, it can be noted that yields up to 8% and 10.8% were achieved using carbon dioxide and propane as solvent, respectively. It should be mentioned that for carbon dioxide as extraction solvent the yield was evaluated after 200 min of extraction, while for propane the extraction yield was measured after 30 min of extraction. These results indicated that propane is a more suitable solvent for grape seed oil than carbon dioxide. These results agree with those obtained by Illés and co-workers (10, 14), who also found that the solvating power of propane was much higher than that of CO₂.

Figure 1 presents the kinetic of extractions of grape seed oil using carbon dioxide as solvent. From this figure, it is clearly noted that density and temperature exert a pronounced effect on the extraction. In experimental conditions 1 to 5, the temperature was fixed at 45 °C and reduced density was increased from 0.83 to 1.85. It is well-known that the solvent

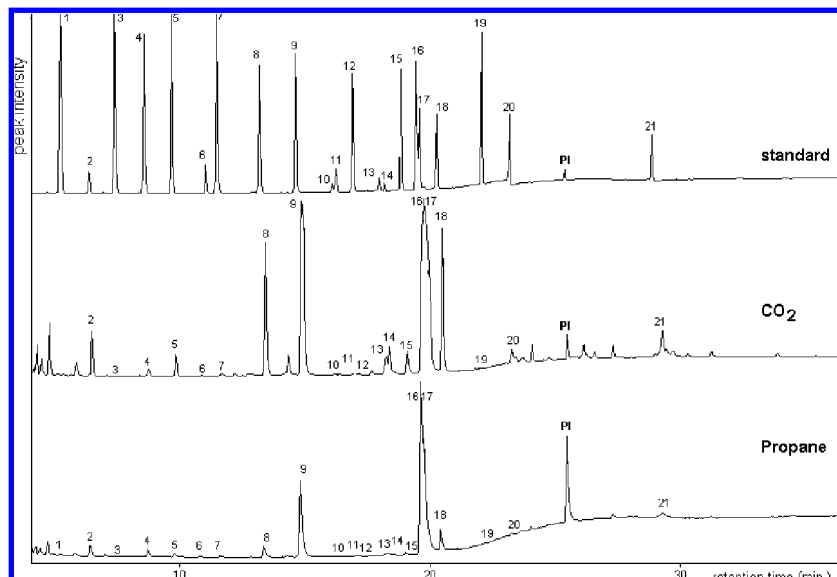


Figure 4. Typical monitored ion chromatogram (MIC) of grape seed oil extracted with carbon dioxide, propane, and a standard solution.

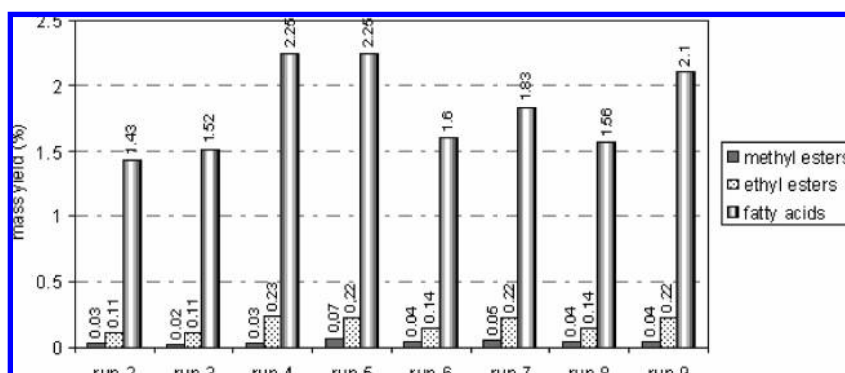


Figure 5. Quantitative analysis of grape seed oil extracted with compressed CO₂ and propane. Components: FFA, free fatty acids; EE, ethyl esters; ME, methyl esters. Runs are labeled according to Table 2.

power of a supercritical fluid is directly related to the fluid density, and in this sense, as the density increases, the extraction yield is enhanced.

Comparing runs 6, 4, and 7, it could be noted that temperature and pressure also exert a positive effect. As the temperature is increased, transport properties (diffusion coefficient and viscosity) of the solvent are favorable, facilitating the penetration of the solvent in the porous vegetable matrix to oil solubilization. Also, higher temperatures enhance the vapor pressure of the oil making easy its extraction by the solvent. An increase in the pressure facilitates the penetration in the pores of the matrix, facilitating the contact of the solvent with the compounds that will be extracted. On the other hand, at constant pressure, the enhancement of temperature promotes a decrease in the solvent density. From Table 2 and Figure 1, it is clearly noted that the effect of temperature must be evaluated at constant density and not at constant pressure in supercritical fluid extraction. For example, when comparing runs 2 and 4 (similar pressure), it seems that temperature presents a negative effect on the extraction. In fact, at this pressure condition, the effect of decreasing the solvent density with the enhancement in temperature is more relevant to the extraction, leading to a decrease in the extraction yield. This is a mistaken interpretation of the results, and then temperature effect must be evaluated at constant density.

Some works in the literature pointed out that the solubility of vegetable oils in propane is higher than that in CO₂ due to

the high percentage of triacylglycerols in the oil (11, 16). CO₂ is selective for compounds of molecular weight smaller than 500 Da. Generally, the solubility decreases with the increase of the molecular weight in a homologous series (28). The composition of grape seed oil is formed mainly of triacylglycerols of fatty acids with 18 carbons atoms in the molecule but also possesses free acid fatty and mono- and diglycerides. Figure 2 presents the kinetic of extraction performed using subcritical propane as solvent compared to the best results obtained with supercritical CO₂ as solvent.

It should be noted from this figure that after 30 min of extraction yields around 10.8% are achieved using propane as solvent. For this time, around 5% of yield was obtained with carbon dioxide. These results evidenced a very fast extraction kinetic obtained with propane and corroborate previous results that propane is a better solvent than carbon dioxide also for the grape seed vegetable oil. From Figure 2, it could also be noted that the temperature does not have a pronounced effect on the extraction yield. The work of Ndiaye et al. (17) indicated that at 100 bar the vegetable oils from soybean and olive are totally miscible with propane in the temperature range investigated in this work. In this sense, no thermodynamic solubility limitations are presented in the conditions investigated, and mass transfer limitations are very low, leading to similar extraction yields.

Qualitative Analysis of Free Glycerol Compounds. The analyses of free glycerol compounds were performed in this work considering fatty acids, methyl esters, ethyl esters, sterols,

and others compounds. **Figure 3** shows examples of a total ion chromatogram (TIC) of the oil extracted with CO₂ and propane, while **Table 3** presents the tentative identification compounds in the extracts. The identification was accomplished by comparison with mass spectra from the library (Wiley) of the equipment.

A general inspection of **Table 3** reveals that samples extracted with propane present a smaller number of free glycerol compounds in the oil than samples extracted with carbon dioxide. This fact could be attributed to the higher hydrophobicity of propane compared to carbon dioxide. In this sense, carbon dioxide could be a more effective solvent to the slight polar compounds like free fatty acids presented in the oil. Also, from **Table 3**, it should be noted that the experimental condition 8 (propane at 30 °C) presents a smaller amount of peaks than at 45 °C (experimental condition 9), suggesting that at lower temperatures the hydrolysis of triacylglycerol molecules to free fatty acid formation was unfavorable. This fact is corroborated by the absence of compounds with glycerol in the experimental conditions at lower temperature (experimental condition 8).

Besides free fatty acid, ethyl, and methyl esters, monoacylglycerols (palmitin, linolein, and olein) were also identified. Diacylglycerols and triacylglycerols should be trapped in the GC-liner and were not identified. **Table 3** also shows that the main sterols found in the grape seed oil were brassicasterols, stigmasterols, and stigmatadienol. Also, the derivatization procedure was not reliable for phenol compounds because both derivatized and underivatized sterols were identified.

Quantitative Analysis for Free Glycerol Compounds. **Figure 4** presents the monitored ion chromatogram (MIC) of the standards and typical samples extracted with CO₂ and propane. The authentic standards utilized for the quantitative analysis of the grape seed oil extracts were presented in **Table 1**. **Figure 5** presents the content of total fatty acid, ethyl, and methyl esters present in the grape seed oil. It should be noted that these minority compounds are present in low concentration in the whole vegetable oil (<3%). **Figure 5** also shows that as the carbon dioxide density is increased the amount of fatty acid extracted is higher. This aspect could be attributed to the higher solvent power of carbon dioxide at higher density, helping the solubility of moderately polar fatty acids present in grape seed oil. An interesting fact occurs when the temperature varies from 30 to 45 °C, where higher temperatures induce larger amounts of fatty acids in the grape seed oil extracted. This aspect also occurs with carbon dioxide from 30 to 45 °C, but at 60 °C, the amount of fatty acid is reduced.

Traditionally, traces of free fatty acids can be analyzed by GC-flame ionization detection (FID) as methyl esters previously derivatized (7). The problem of this technically is the incorrect concentrations of free fatty acids, as part of these compounds are in fact original esters presented in the oil. The trimethylsilyl derivatization allowed the identification of real free fatty acids and the original esters in the sample. The samples extracted with propane and CO₂ presented a similar composition of esters. These compounds can react forming a larger concentration of free fatty acids present in vegetable oils. The high content of polyunsaturated fatty acids in grape seed oil should also be pointed out. Even at higher-temperature extraction with carbon dioxide, there was no deterioration of linoleic and oleic acids, probably due to the presence of natural antioxidants such as tocopherols, sterols, and phenolic compounds (19, 22). The quantitative analysis of major compounds did not reveal great differences among the extracts obtained with CO₂ and with propane.

From the results obtained, it could be pointed out that propane is a more suitable solvent for grape seed oil extraction yield than carbon dioxide, as higher extraction yields and faster kinetics of extraction were achieved with this solvent. This fact was attributed to the higher solubility of vegetable oils in propane than in carbon dioxide. In relation to compressed carbon dioxide extractions, both temperature and density showed a remarkable and positive effect on the extraction yield. The results showed that fatty acid, ethyl, and methyl esters are present in low concentration in the whole vegetable oil (<3%) and that, in general, samples extracted with propane present a smaller amount of peaks of free glycerol compounds in the oil than samples extracted with carbon dioxide. Temperature, pressure, and density of extraction also present a positive effect on the quantities of fatty acids in the extracted oil.

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