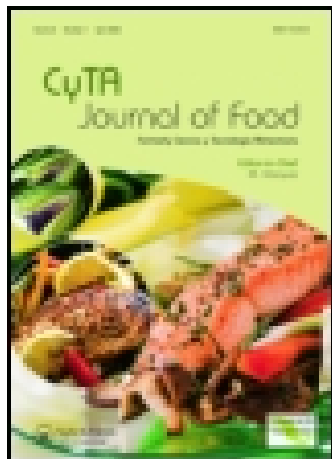


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ARTICLES

Grape by-products: extraction of polyphenolic compounds using supercritical CO₂ and liquid organic solvent – a preliminary investigation

Subproductos de la uva: extracción de compuestos polifenólicos usando CO₂ supercrítico y disolventes orgánicos líquidos – una investigación preliminar

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This research focussed on the exploitation of grape by-products as a source of polyphenolic compounds, which are of interest to the food, pharmaceutical and cosmetic industries. In particular, two substrates were tested: Pinot Noir grape skins and grape seeds. Pinot Noir grape skins were extracted by supercritical CO₂ added with ethanol as modifier at constant temperature (45 °C) and at variable pressure (200, 300, 400 or 500 bar). The supercritical extraction kinetics of polyphenolic compounds was obtained. Grape seeds were extracted by combining supercritical (at 40 °C and 500 bar and using CO₂ or CO₂ added with ethanol as modifier) with liquid ethanol extraction. The supercritical technique seemed not to be really effective in extracting polyphenolic compounds; it can be anyway utilized to selectively extract grape seed oil by avoiding any solvent contamination of the matrix which can be further extracted for the recovery of polyphenolic compounds by means of liquid organic solvent.

Keywords: grape by-products; phenolic compounds; extraction; kinetics; supercritical extraction; solvent extraction

Esta investigación se enfocó en el aprovechamiento de subproductos de la uva como una fuente de compuestos polifenólicos, los cuales son de interés para la industria alimentaria, farmacéutica y cosmética. En particular, dos sustratos se estudiaron: pieles y semillas de uva de Pinot Noir. Las pieles se extrajeron con CO₂ supercrítico adicionado con etanol como modificador a temperatura constante (45 °C) y a presión variable (200, 300, 400 o 500 bar). Se obtuvo la cinética de la extracción supercrítica de compuestos polifenólicos. Las semillas de uva se extrajeron combinando supercrítico (a 40 °C y 500 bar usando CO₂ o CO₂ adicionado con etanol como modificador) con extracción en etanol líquido. La técnica supercrítica parece que no es realmente efectiva para la extracción de compuestos polifenólicos; puede ser de todas formas usada para extraer selectivamente aceite de semilla evitando contaminaciones de la matriz con cualquier disolvente, que puede ser posteriormente extraído para la recuperación de compuestos polifenólicos por medio de disolvente orgánico líquido.

Palabras clave: subproductos de la uva; compuestos polifenólicos; extracción; cinética; extracción supercrítica; extracción con disolventes

Introduction

Every year, the world wine industry processes about 35,000 million kg of grape and produces about 7000 million kg of grape by-products: grape pomace consisting of skins, seeds and stalks (Pinelo, Arnous, & Meyer, 2006). This grape marc is mainly utilized by the distillery industry to produce alcohol and alcoholic drinks; a similar amount of by-products (about 4500 million kg) results from this industry in the form of spent marc constituted by fermented seeds and skins. Also these by-products have a market: oil is extracted from grape seeds, whereas grape skins are utilized in animal feed formulations for their high protein content. In some cases, they also find application as biomass for energy

production. Moreover, other minor utilizations take place: compost production, recovery of tartrates, additives and natural colours. Because both grape and grape-derived products are very rich in antioxidant compounds, depending on the grape variety, the vine system and other factors (Alén-Ruiz, García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2009; García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2007; Pérez-Lamela, García-Falcón, Simal-Gándara, & Orriols-Fernández, 2007), recent applications focus on their recovery: the cosmetic and pharmaceutical industries sell, for example, grape seed extracts and beauty creams, whose antiradical activity is due to the antioxidant compounds extracted from

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vegetable matter, such as grape seeds (and also blueberries, officinal plants, etc.).

In a previous work, grape seed oil extraction was considered (Fiori, 2007). Now, the exploitation of grape by-products as a source of polyphenolic compounds, which are well known for their antioxidant properties, was studied. The literature presents a fairly good number of works dealing with the extraction of these compounds from grape seeds, skins and also stems. Most of them consider the liquid solvent extraction technique, some the supercritical one. Some others deal with the thermodynamic properties (and in particular the solubility) of these compounds in supercritical CO₂. Without any doubt, the interest on the topic has recently increased, following today's concern about healthy constituents of food and about healthy industrial procedures to obtain these constituents.

The first scientific work on supercritical extraction of the phenolic compounds typical of grape residues was performed by Tena, Rios, and Valcarcel (1998). Palma and Taylor (1999) were the first to apply supercritical extraction of grape seeds in order to recover polyphenolic compounds. Murga, Ruiz, Beltran, and Cabezas (2000) reported on the extraction of complex phenols and tannins from grape seeds by means of supercritical CO₂ added with modifier (ethanol and methanol). Pascual-Marti, Salvador, Chafer, and Berna (2001) extracted grape skins by CO₂ modified with ethanol, and they evaluated their resveratrol content. Arce, Lista, Rios, and Valcarcel (2001) and Palenzuela, Rodriguez-Amaro, Rios, and Valcarel (2002) developed different systems for the on-line screening of polyphenols extracted from grape marc by supercritical fluid; various cultivars were tested and the total content of polyphenols resulted in the range 81–192 mg/kg (Palenzuela et al., 2002). Louli, Ragoussis, and Magoulas (2004) presented a procedure to recover phenolic antioxidants from grape marc; the process involved two basic steps: liquid solvent extraction and purification of the dried extract by means of supercritical CO₂. Ashraf-Khorassani and Taylor (2004) extracted Chardonnay grape seeds by means of three different steps in the following sequence: supercritical pure CO₂ was used to remove the oil, then 40% methanol-modified CO₂ was used for the extraction of monomeric polyphenols, finally pure methanol at high pressure was used to recover polyphenolic dimers and trimers and procyanidins. Chafer, Pascual-Marti, Salvador, and Berna (2005) analysed supercritical fluid extraction of polyphenolic compounds from spiked inert solid and from the dried skin of five grape varieties. Pinelo et al. (2007) in their comparative study extracted distilled white grape pomace by means of solid–liquid extraction and supercritical extraction; the extracts obtained with the former technique contained more proanthocyanidins, the ones obtained with the latter technique basically contained gallic acid, catechin and epicatechin.

The goal of this research was to test the capability of supercritical extraction of polyphenolic compounds

from grape marc, and the attention focussed on two main aspects:

- (1) the characterization of supercritical extraction kinetics of polyphenolic compounds from grape skins;
- (2) the evaluation of the combination of supercritical and liquid organic solvent extraction to obtain polyphenolic compounds from grape seeds.

According to what we know, the first aspect represents a novelty in the literature: usually authors refer to global extraction yield when dealing with supercritical extraction of polyphenolic compounds, and there is no available information regarding the extraction kinetics. This information is of fundamental importance, for instance, for process modelling and optimization. The second aspect focusses on the possible synergy between the two different extraction techniques and, hopefully, could be of some interest for future industrial applications.

Given its low polarity, CO₂ was added with ethanol as modifier in the supercritical experiments. Ethanol and methanol were utilized as liquid organic solvents.

Pinot Noir grape skins were used for the extraction kinetics characterization due to their high content in polyphenolic compounds. Moreover, the potential importance for the industrial reutilization of Pinot Noir grape marc as a source of antioxidants derives from the large use of this cultivar in the white wine-making process; the latter, unlike the red wine-making process, does not modify to a large extent the polyphenolic contents of grape skins and seeds.

Materials and methods

Grape marc and seeds

Pinot Noir grape marc was collected by a Trentino wine-making company just after wine-pressing (vintage 2006). The marc, composed of skins, seeds and stalks, was rapidly carried to our laboratories, frozen and maintained at –20 °C.

Dried grape seeds were kindly provided by a Trentino distillery. They were divided (and sold) by the distillery according to two different typologies: dried grape seeds from fermented grape marc and non-fermented grape marc. The latter category was mainly composed of Chardonnay and Pinot Gris grape seeds, whereas the composition of the former presented great variations. Dried grape seeds maintained unchanged properties for months (according to the distillery, for years).

Sample preparation for extraction and dimensional characterization

The grape marc with a moisture content of 60–70% was dried to a moisture content of 4–5% in an oven at 65 °C. The dried marc was separated into seeds, skins

and stalks by sieving. The skins were milled in a blender. After the extraction, the particle size distribution was checked by sieving the matrix with a vibrating screen. Table 1 shows the granulometric distributions for the tests of Figure 2 – see later; in the same table, the Sauter mean diameter, a common reference dimensional value when dealing with solvent extraction

Table 1. Granulometric distributions of milled grape skins relevant to tests reported in Figure 2.

Tabla 1. Distribuciones granulométricas de las pieles molidas de uva, relevantes a las pruebas reportadas en la Figura 2.

Mesh size (mm)	Retained mass percentage (%)			
	500 bar	400 bar	300 bar	200 bar
4.50	0.00	0.00	0.00	0.00
3.55	0.00	0.00	0.00	0.00
2.36	0.38	1.03	0.19	0.19
2.00	0.96	2.26	1.67	1.12
1.70	1.34	4.12	0.74	1.68
1.40	2.68	6.79	2.22	2.23
1.18	4.60	8.85	3.70	3.35
1.00	6.13	10.08	5.00	4.84
0.85	7.85	10.29	7.04	6.52
0.71	9.77	10.91	9.44	8.75
0.60	10.73	11.32	10.74	10.61
0.50	9.20	7.20	9.44	9.12
0.425	7.85	5.76	9.26	8.57
0.355	9.39	6.17	10.37	9.87
0.30	6.32	3.91	7.22	7.26
0.25	6.32	3.29	6.85	6.89
0.20	6.13	3.09	6.48	6.89
0.15	4.21	2.06	4.07	4.66
0.10	3.64	1.65	3.52	4.47
0	2.49	1.23	2.04	2.98
Sauter mean diameter (mm)	0.38	0.55	0.39	0.35

processes, was also reported. The particle Sauter mean diameter for the experimental runs was in the range 0.35–0.55 mm.

The grape seeds were provided dried, with moisture level of about 5%. Before supercritical extraction, they were milled in a blender. Before extracting the whole grape seeds with organic solvent, they were manually milled to a fine powder, by means of a pestle and mortar.

Reagents

The liquid CO₂ (purity >99.99%) used as supercritical solvent was purchased from Rivoira (Italy) in cylinders containing 30 kg of product. HPLC-grade ethanol, hexane and methanol for extraction were purchased from Fluka (USA).

The extractor

The facility (PRORAS[®]) consisted of a typical supercritical extraction equipment. Figure 1 shows a simplified P&ID of the system.

Liquid CO₂ contained in a pressure bottle was under-cooled in a heat exchanger by a glycolic solution kept at low temperature by a refrigeration cycle. An air-driven piston pump (Haskel 2.0 HP-122), whose head was cooled by the same refrigerant solution, increased the solvent pressure. An HPLC pump (Gilson Piston Pump, model 307) increased the co-solvent pressure. The two high-pressure liquids were mixed before being heated in a heat exchanger with a dedicated temperature control loop. The fluid passed through the extractor vessel, inside which a basket with an internal volume of 0.1 l held the substrate to be extracted. The basket was a metal cylinder closed on both ends by removable metal frits, whose function

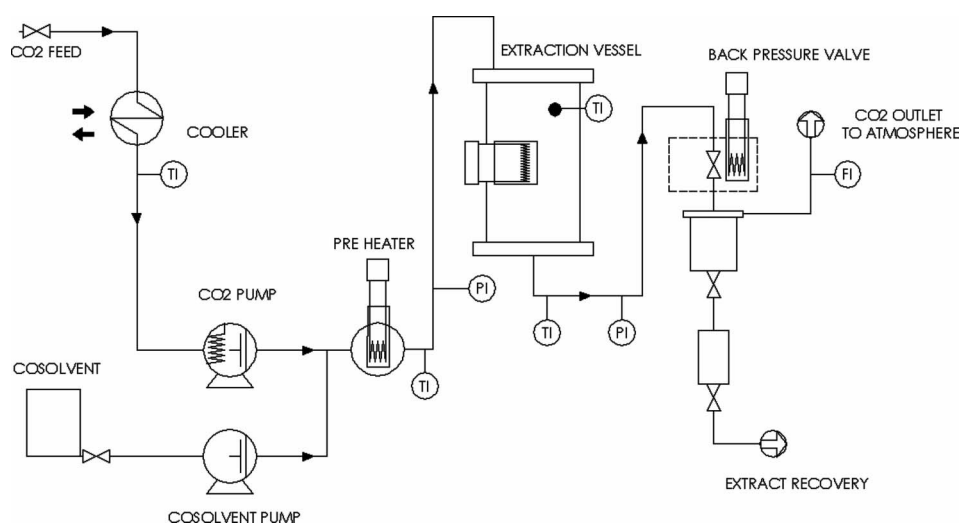


Figure 1. P&ID of the extraction equipment.

Figura 1. P&ID del equipo de extracción.

was to homogeneously distribute the solvent (upper frit) and to act as a basis for the material inside the cylinder (lower frit). A detailed description of the basket assembly geometry was reported elsewhere (Fiori, 2007); referring to the description reported in that work and considering that the assembly geometry affects the extraction course, it is important here to underline that, by means of a tailor-made spacer, the 0.1-l basket was placed at the bottom of the extraction vessel (internal volume: about 0.5 l) to avoid the presence of void volume below the basket itself. Finally, CO₂ expanded into a glass vessel where the extracted substances were collected. The temperature of the extractor vessel was monitored and controlled. The pressure was maintained at the desired value by adjusting a heated manual metering valve at the extractor outlet. The CO₂ flow rate, which resulted from the pump frequency and which could be regulated by varying the pump motive air pressure, was measured by a thermal mass flow meter (KOBOLD[®] MAS-6017-V2-A) placed behind the collecting vessel in the atmospheric CO₂ discharge line. The CO₂ flow rate was totalized and recorded during the experiments. The CO₂ bottle was supported by a weighing platform allowing to follow its emptying.

Polyphenols from grape marc

The aim of this investigation was to characterize the supercritical extraction kinetics of polyphenolic compounds from Pinot Noir grape skins. The extraction temperature was maintained constant for the various tests, whereas the pressure was varied in the range 200–500 bar. For each test, about 50 g of grape dried skins were extracted.

The milled substrate was filled in the extraction basket that was then charged inside the extraction vessel. The vessel temperature was set to a constant value of 45 °C and the vessel was pressurized to the selected level maintaining the pressure regulating manual valve in the close position. Once the desired pressure was achieved, the co-solvent pump was started and regulated to flux 0.90 ml/min of ethanol (modifier). During this “static” extraction time, lasting 30 min, the pressure of the system naturally increased, due to the co-solvent addition. As soon as the static extraction ended, the pressure-regulating manual valve was opened (“dynamic” extraction) to allow the desired outlet solvent flow: the pressure of the vessel naturally lowered to a value close to the desired one, which was precisely fulfilled regulating the motive pressure to the CO₂ air driven pump. During the static extraction time, about 27 ml of ethanol entered the vessel. Considering the values of the CO₂ density (<http://www.nist.gov/srd/nist23.htm>) at the tested operative conditions, the ethanol concentration at the beginning of the “dynamic” extraction resulted in the range 0.054–0.064 ml EtOH/g CO₂.

Table 2 reports the distinctive conditions for the various tests: the CO₂ flow rate, the pressure, the

amount of substrates charged (Pinot Noir grape skins) and the polyphenols extraction yield expressed in terms of gallic acid equivalents (GAE) – see the sections “Analytical methods” and “Polyphenols from grape seeds”. The temperature and the ethanol flow rate were maintained the same for the various tests, and equal to 45 °C and 0.90 ml/min, respectively. The average value of the solvent flow rate (16 g/min) corresponded to an inlet ethanol concentration of 0.056 ml EtOH/g CO₂.

During the gas expansion, some ethanol vaporized and some ethanol condensed as liquid phase. The latter, containing the extract, was withdrawn every 30 min from the glass expansion vessel and collected into a vial. In sequence with the ethanol removal, the glass vessel was cleaned with 10 ml of ethanol to recover extracted compounds stuck to the glass vessel. This ethanol was collected into another vial. By this procedure, for a test duration of 5 h, 20 vials were collected (two representative of each extraction step lasting 30 min). Then the test ended: the co-solvent pump and the CO₂ pump were stopped and the system was allowed to depressurize to atmospheric pressure. Some quantity of ethanol resulted from the depressurizing phase: this ethanol was collected into a vial. The vials were then analysed to evaluate their polyphenolic content.

Polyphenols from grape seeds

To evaluate the effect of combining supercritical extraction with liquid organic solvent extraction on polyphenolic compounds recovery from grape seeds, different milled grape seeds extraction paths were followed:

- (1) extraction with organic solvent;
- (2) extraction with supercritical CO₂, followed by extraction with organic solvent;
- (3) extraction with supercritical CO₂, then with supercritical CO₂ charged with modifier and finally with organic solvent;

Table 2. Supercritical extraction of Pinot Noir grape skins.

Tabla 2. Extracción supercrítica de pieles de uva Pinot Noir

Pressure (bar)	Skin mass (g)	CO ₂ flow rate (g/min)	Yield (mg GAE/100g skin)
500	55.2	17.13	23.57
400	52.5	15.93	28.12
300	54.9	15.64	23.91
200	54.9	16.16	19.83

Experimental operative conditions (temperature = 45 °C, ethanol flow rate = 0.90 ml/min for all the tests) and yield of polyphenolic compounds.

Condiciones experimentales de operación (temperatura = 45 °C, razón de flujo de etanol = 0.90 ml/min para todas las pruebas) y el rendimiento de compuestos polifenólicos.

- (4) extraction with supercritical CO₂ charged with modifier, followed by extraction with organic solvent.

Also in this case, ethanol was selected as modifier. Supercritical extraction provided two kinds of extracts: oils and ethanolic solutions, as well as the high-pressure extracted matrices.

Oils and ethanolic solutions were characterized for their content in polyphenolic compounds. The extracted matrices were further extracted with organic solvent (ethanol), and also these extracts were characterized for their content in polyphenolic compounds.

All the supercritical extractions were carried out at 500 bar and 40 °C. For each supercritical run, about 50 g of grape seeds were extracted.

Seeds from both the red and the white wine-making processes were extracted with organic solvent. Given the extremely low polyphenolic content of the seeds from the red wine-making process (fermented seeds), only the seeds from the white wine-making process (unfermented seeds) were subjected to the various aforementioned extraction paths.

Supercritical fluid extraction

The experimental procedure was similar to the one detailed in the section “Polyphenols from grape marc”. Considering the various extraction paths analysed, some additional details are necessary. Table 3 summarizes the experimental conditions of the three performed tests: pressure, temperature, amount of substrate to be extracted, duration of the different steps, CO₂ and modifier flow rates.

Test 1 was characterized by a flow of supercritical CO₂ for a duration of 1 h and 30 min; Test 2 by a flow of CO₂ for a duration of 1 h, followed by a flow of supercritical CO₂ added with modifier for further 2 h and 30 min; Test 3 by a flow of supercritical CO₂ added with modifier for a duration of 3 h.

Test 1 was stopped when it was detected that no more oil was extracted from the matrix, and, similarly, for Test 2, it was decided to start adding modifier when the oil

Table 3. Supercritical extraction of grape seeds.

Tabla 3. Extracción supercrítica de las semillas de uva.

Test	Seed mass (g)	CO ₂ flow (min)	Ethanol flow (min)	CO ₂ flow rate (g/min)	Ethanol flow rate (ml/min)
1	47.5	90	–	18.03	–
2	50.7	60	–	18.48	–
		150	150	17.07	0.90
3	57.2	180	180	17.48	0.90

Experimental operative conditions (temperature = 40 °C, pressure = 500 bar for all the tests).

Condiciones operativas experimentales (temperatura = 40 °C, presión = 500 bar para todas las pruebas).

being extracted was negligible. Test 1 provided extracted oil, Test 2 extracted oil and, in the subsequent step, an ethanolic extract. During Test 3, the oil was extracted while fluxing ethanol: however, the two liquid phases, having different densities, naturally separated after being collected.

Organic solvent extraction

Organic solvent extraction was carried out at room temperature using ethanol in 20 ml glass test tubes for ~20 h to guarantee maximum extraction efficiency. Hence, 2 g of sample were extracted with 10 ml of solvent and the mixtures were continuously agitated by magnetic stirring in the dark. After extraction, the samples were centrifuged at 7500 rpm for 10 min by a multi-speed centrifuge. Clear extracts were analysed.

Oil polyphenolic compounds extraction

A defined amount of oil (3–5 g) was dissolved in 10 ml hexane. The obtained solution was extracted three times with 12.5 ml of methanol/water (60:40, vol/vol), each time being stirred over a magnetic plate for 10 min and then separated into the two phases by centrifugation (3000 rpm for 10 min). The three hydroalcoholic extracts were then combined and washed twice with 15 ml of hexane. Finally, the hydroalcoholic extracts were evaporated by vacuum at a temperature below 30 °C and then dissolved in 1 ml of methanol before analysis.

Analytical methods

The concentration of total polyphenols (TP) in the extracts was determined with Folin Ciocalteu reagent according to the procedure introduced by Gutfinger (1981). The calibration curve was made with standard solutions of gallic acid in the range 0.01–1.00 mg/ml and the results were expressed as GAE (μ g GAE/ml of extract or mg GAE/100 g of substrate); measures were carried out at 725 nm using an UV–vis spectrophotometer, model Lambda 25 (Perkin Elmer, Wellesley, MA). All analyses were performed in triplicate. The method response was described by the linear equation:

$$ABS_{725} = 0.0017 \cdot TP - 0.0241$$

with $R^2 = 0.9940$. In the above equation ABS_{725} is the absorbance at 725 nm and TP is the concentration of total polyphenols (μ g GAE/ml).

Results and discussion

Extraction from grape marc

Table 4 shows an example of the analytical results: in particular, the data are relevant to the test performed

at 400 bar. The time of extraction, the sample number, the polyphenols concentration, the ethanolic sample volume and finally the polyphenolic yield were reported in the various columns.

The same analytical results (yield) and the ones relevant to the tests performed at 500, 300 and 200 bar were reported in Figure 2: the amount of polyphenolic compounds (expressed as mg GAE per 100 g of substrate) were plotted *versus* the CO₂ consumption. It was evident that the concentration of the poly-

Table 4. Supercritical extraction of Pinot Noir grape skins.

Tabla 4. Extracción supercrítica de las pieles de la uva Pinot Noir.

Time (min)	Sample	Conc (µg GAE/ml)	Sample volume (ml)	Yield (mg GAE/100g skin)
30	1	307.72	6.5	7.170
	2	153.38	11.5	
60	3	163.85	14.0	5.882
	4	79.42	10.0	
90	5	126.40	10.0	3.868
	6	73.02	10.5	
120	7	111.93	8.0	2.618
	8	47.92	10.0	
150	9	67.93	10.5	1.845
	10	25.52	10.0	
180	11	54.34	11.0	1.484
	12	19.09	9.5	
210	13	57.23	10.0	1.544
	14	23.84	10.0	
240	15	40.44	10.0	1.246
	16	24.95	10.0	
270	17	41.35	10.0	1.239
	18	23.69	10.0	
300	19	32.78	9.0	0.837
	20	14.43	10.0	
Depress.	21	34.19	6.0	0.391
Total				28.12

Analytical results for the test conducted at 400 bar.

Resultados analíticos para la prueba conducida a 400 bar.

phenolic compounds decreased rapidly during the extraction time. Moreover, a trend with pressure seemed clear: at the beginning of the extraction, the higher the pressure, the higher the concentration. But, for each test, the yield in polyphenolic compounds seemed rather low – Table 2 – and at 400 bar it was the greatest. The extraction kinetics seemed to be correlated to a matrix depletion in extractable polyphenolic compounds. The yield values here obtained – 20 to 28 mg/100 g – were in good agreement with those obtained by Arce et al. (2001) – 13 to 30 mg/100g – and by Palenzuela et al. (2002) – 8 to 19 mg/100g – who extracted grape marc, but lower than those obtained by Chafer et al. (2005) – 95 to 130 mg/100g – who extracted grape skins. Besides, the yield values were quite low if compared to liquid organic solvent extraction (Baydar, Ozkan, & Sagdic 2004), because of the incapability of the modified supercritical solvent to extract high-molecular weight polyphenols, such as oligo-procyanidines, being instead selective for compounds such as gallic acid, catechin and epicatechin (Ashraf-Khorassani and Taylor, 2004; Pinelo et al., 2007).

Extraction from grape seeds

Preliminary organic solvent extractions were performed on both fermented and non-fermented grape seeds. The former resulted very poor in polyphenolic compounds, the latter quite rich (the yield being equal to 147 and 4080 mg/100g, respectively – Figure 3). The values of polyphenolic yield from seeds found in the literature are the following: 380 mg/100g (Guendez, Kallithraka, Makris, & Kefalas, 2005); 1472–6681 mg/100g (Bucic-Kojic, Planinic, Tomas, Bilic, & Velic, 2007); 66,700 mg/100g (Baydar et al., 2004). It is important to underline that the latter value was obtained extracting seeds from grape cluster, not previously industrially processed as in the former (and in the present) cases.

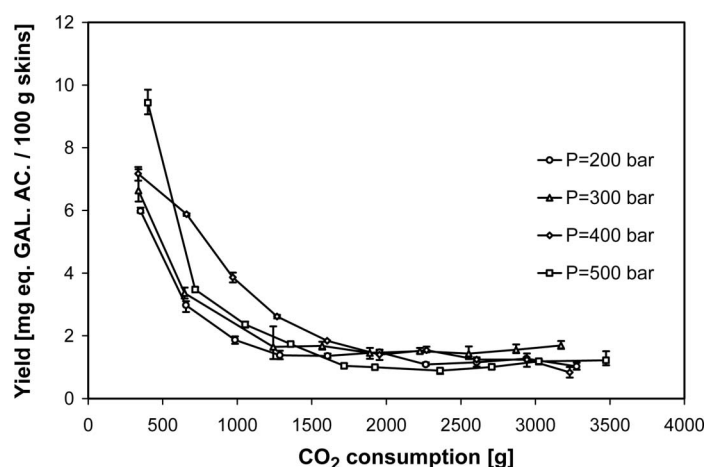


Figure 2. Supercritical extraction of polyphenolic compounds from Pinot Noir grape skins: extraction kinetics.

Figura 2. Extracción supercrítica de compuestos polifenólicos de las pieles de uva Pinot Noir: cinética de la extracción.

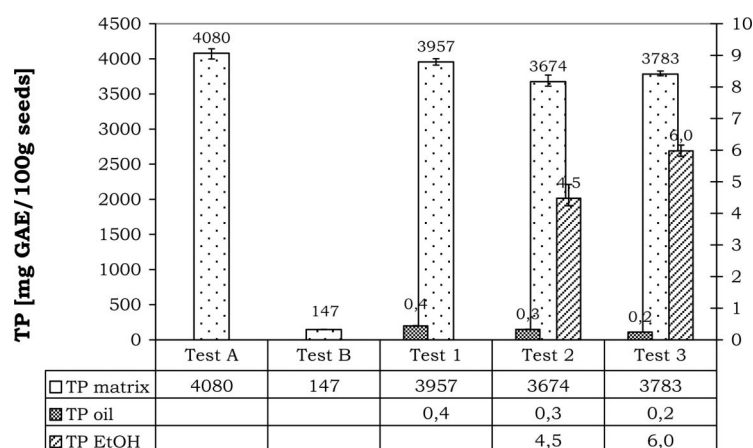


Figure 3. Extraction of polyphenolic compounds from grape seeds: analytical results.

Figura 3. Extracción de compuestos polifenólicos de las semillas de la uva: resultados analíticos.

Consequently, the attention was focussed only on non-fermented grape seeds. They were extracted according to the procedures reported in section “Polyphenols from grape seeds”. Figure 3 reports the analytical results for the different experimental tests: besides, the polyphenolic yield resulting from organic solvent extraction (Test A: unfermented seeds; Test B: fermented seeds) and the polyphenolic yield for each fraction resulting from supercritical extraction were evidenced (Tests 1, 2 and 3).

Three main results seemed noteworthy:

- (1) The substrate was to a marginal extent depleted in polyphenolic compounds by the high-pressure extraction. Considering Test 1, it was confirmed that pure CO₂ was incapable of extracting polar compounds such as the ones in analysis: the matrix depletion was negligible. The addition of the modifier favoured an increased polyphenols extraction, but the maximum matrix depletion remained quite low (about 10%, Tests 2 and 3).
- (2) The analyses revealed absolutely trivial amounts of polyphenolic compounds in the grape seed oil. This was true both for the oil extracted in the presence of the only CO₂ (Tests 1 and 2) and for the oil extracted with CO₂ plus modifier (Test 3). For the latter case, the polyphenolic compounds probably tended to solubilize in the ethanolic liquid fraction in the expansion glass vessel. However, also in this fraction the amount of these compounds was negligible (Tests 2 and 3).
- (3) The polyphenolic mass balance did not seem to be respected: the amount of polyphenolic compounds removed from the substrate did not correspond to the content of the same substances in both the oil and the ethanolic fraction. Hypothetically, this aspect could be linked to

the thermochemical instability of polyphenolic compounds: their concentration in the extracted samples could, in fact, decrease with time because of oxidative and thermal degradation phenomena; however, this appeared unlikely according to our findings (not reported data): in the laboratory storage conditions, the polyphenolic compounds in ethanolic solutions were quite stable. Most probably, the reason lied on the standard error of the TP measurement which could be estimated in $\pm 10\%$: taking into consideration this aspect, the fact that the mass balance was not respected lost physical significance, while the single measured values (valid with an approximation of about $\pm 10\%$) maintained their validity.

Moreover, the polyphenol content of grape seed oil, expressed as micrograms per gram of oil ($\mu\text{g}/\text{g}_{\text{oil}}$), was found to be in the range 21–66. Such values were one order of magnitude higher than that reported by Colquhoun, Hicks, and Reed (1996) ($2 \mu\text{g}/\text{g}_{\text{oil}}$). However, these data seemed to confirm the reduced polyphenol content of grape seed oil when compared to that of olive oil, for which values up to $1700 \mu\text{g}/\text{g}_{\text{oil}}$ were reported (Beardsell, Francis, Ridley, Robards, 2002).

Conclusion

In this study, the extraction of polyphenolic compounds from grape by-products by utilizing supercritical CO₂ (pure or mixed with co-solvent to increase its polarity), liquid organic solvent and a combination of the two was investigated.

Pinot Noir grape marc was selected according to its relatively large content in the compounds of interest. After preliminary analyses on fermented and unfermented grape seeds, the latter were chosen for their considerably larger amount of polyphenols.

By using supercritical CO₂ with ethanol as modifier, the polyphenolic compounds extraction kinetics from grape marc turned out: for all the analysed pressures (200, 300, 400 and 500 bar), the amount of extracted compounds decreased with time, pointing out a matrix depletion in extractable polyphenolic compounds. The final yield was always rather low, but in good agreement with literature data.

Extracting grape seeds in various sequences with supercritical CO₂, supercritical CO₂ plus modifier and organic solvent at atmospheric pressure evidenced that the supercritical technique (at least for the operative conditions selected here) was not really effective in extracting polyphenolic compounds. The analyses of both the oil and the ethanolic extracted fractions showed negligible amount of polyphenols. Correspondingly, the high-pressure extracted matrices resulted only partially depleted in polyphenols.

We are aware that the presented results have to be considered as preliminary ones, but at the same time we are confident that they have a qualitative and quantitative value. In particular, the following points can be concluded:

- (1) to the best of our knowledge, for the first time supercritical extraction kinetics of polyphenolic compounds from grape skins was characterized; the low values of extraction yield do not depend on reduced extraction time, but on the low CO₂ affinity (even if added with ethanol) for polyphenolic compounds;
- (2) the different extraction paths used to evaluate the effect of combining supercritical with liquid organic solvent extraction confirmed the lower affinity of supercritical CO₂ for polyphenolic compounds with respect to organic solvents at atmospheric pressure. If, as it seemed, supercritical extraction is not the most valuable technique to obtain polyphenolic compounds from grape by-products, it can be anyway utilized to selectively extract grape seed oil, thus avoiding any solvent contamination of the matrix, which can be successively extracted by liquid organic solvent for polyphenolic compounds. In this way, two valuable products are obtained from the same substrate.

Finally, it is to be stressed that a deeper analysis of this potential extraction path is necessary for the optimal exploitation of grape by-products and the subsequent industrial application, whereas the other tested combinations were demonstrated to be not convenient (i.e. extraction with supercritical CO₂ charged with modifier, followed by extraction with organic solvent).

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