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Grape seed oil extraction: Interest of supercritical fluid extraction and gas-assisted mechanical extraction for enhancing polyphenol co-extraction in oil

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ABSTRACT

The aim of this study is to compare three oil extraction methods and to evaluate their efficiency for producing an oil rich in polyphenols. The three extraction methods are screw pressing, extraction by supercritical CO_2 percolation and the combination of these two processes (Gas-Assisted Mechanical Expression: GAME). Screw pressing is the most efficient process for producing grape seed oil with a high yield, but supercritical CO_2 process permits an increase of polyphenol co-extraction with oil. The GAME process allows extraction of more polyphenols than screw pressing and constitutes an interesting process considering oil yield.

RÉSUMÉ

L'objectif de cette étude est de comparer trois procédés d'extraction et d'évaluer leur efficacité pour produire une huile riche en polyphénols. Les trois procédés étudiés sont le pressage à vis, l'extraction par percolation au CO_2 supercritique et la combinaison du pressage et de cette extraction : le pressage mécanique assisté par fluide supercritique (PAFSC). Le pressage à vis est le procédé le plus efficace en termes de rendement en huile, alors que l'extraction par CO_2 supercritique permet une augmentation de la co-extraction de polyphénols dans l'huile. Le PAFSC conduit à une augmentation de la co-extraction des polyphénols en comparaison du pressage à vis, et constitue ainsi un procédé alternatif intéressant pour la production d'huile.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the major crop produced worldwide (66 million tons in 2009 [1]). Its utilizations include fruit consumption, pharmaceutics and wine making (from 70 to 80%). Residue of wine making is named grape pomace and accounts for 20% of grape (w/w). It is composed of seeds, 38 to 52% on a dry matter basis, but also of stems, pulps and skins [2]. The presence of oil and the high phenolic content of grape seeds offers alternative valorization pathways for these by-products [3].

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Grape seed contains from 8 to 20% of oil (dry basis), which is mainly composed of unsaturated fatty acids (linoleic and oleic fatty acids: 58 to 78% and 10 to 20%, respectively [4,5]). Grape seed oil has an unusual high smoking point (190–230 °C, according to Morin [6]) due to the presence of saturated fatty acids (10%). Additionally, this oil is reported to contain minor bioactive components such as phenolic compounds (between 59 to 360 mg Gallic Acid Equivalent [GAE]/kg) [7,2]. Polyphenols identified in grape seed are catechin, epicatechin, trans-resveratrol and procyanidin B1 [2,8]. These phenolic compounds are reported to be involved in a wide range of biological activities [9], but are mostly known for their antioxidant properties. Given the unsaturation level of grape seed oils, those compounds are beneficial for oil conservation [9]. The grape seed oil extraction method does not affect the fatty acid profile considering solvent extraction (hexane or petroleum ether) and Supercritical Fluid Extraction (SFE) [10]. The quality of grape seed oil (free fatty acid, iodine index, saponification index, unsaponifiable fraction, peroxide index, and fatty acid composition) extracted by SFE is similar to that of oil extracted by organic solvent and then refined according to Molero Gómez et al. [11]. On the other hand, total phytosterol extraction was higher with SFE than with petroleum ether [12].

At an industrial scale, oil contained in oilseeds is commonly extracted by screw pressing, which is often followed by organic solvent extraction steps to enhance the global oil yield. However, in the case of seeds with a low oil content such as grape seeds, solvent extraction is preferably used to maximize oil extraction yield [13]. Although high oil yields are achieved by this process, use of organic solvents has several limitations, among which:

- the environmental toxicity;
- the fluctuating price of solvent accordingly to petroleum;
- the non-selective solubility towards lipophilic compounds [14].

Apart from mechanical (screw pressing or hydraulic pressing) and solvent processes, alternative oil extraction technologies focus mainly on water processes (enzymatic) and supercritical fluid extraction. The latter has been extensively studied using supercritical CO_2 on different raw materials (e.g., linseed [15], rapeseed [16], grape seeds [11,17,18]). The oil yield can be maximized according to processing parameters, among which CO_2 pressure and temperature. However, to reach high oil yields, intensive pressure has to be applied due to increase of the solvent power of CO_2, inducing an increase of operating costs [19]. Within an objective of reduction of cost and energy, an alternative expression process was developed: Gas-Assisted Mechanical Expression (GAME) [20,21]. The principle of GAME relies notably on a partial displacement of oil by CO_2 during the pressing, resulting in an increase of oil yield [22]. Studies of this batch process include at first a step of seed conditioning in supercritical CO_2 followed by oil expression under uniaxial compression. The use of a continuous flux of CO_2 during expression would then be another step towards an industrialization of this process.

In this work, the GAME process under a continuous flux of supercritical CO_2 is evaluated. The objective of this study is to compare oil yield and total polyphenol content of oils extracted by GAME, supercritical fluid extraction without pressing (SFE) and screw pressing as a reference process.

2. Experimental procedures

2.1. Grape seed processing

2.1.1. Raw material

Grape seeds were provided by the Distillerie Jean-Goyard (Aÿ, France). The duration between grape pressing and air drying of grape seeds was 15 days. Their moisture content was lowered to about 7% (db) by air drying at the Distillerie. Oil and water content of the seeds were determined according to French standard procedures [23,24], respectively. The studied grape seed are composed of 12.2 ± 0.5% of oil (db: dry basis) and 6.78 ± 0.03% of water (db).

The material was kept in a closed bag, at room temperature, until processing. For SFE and GAME experiments, grape seeds were grounded using a knife mill (Urshel, USA). The particle size (70%) was comprised between 600 and 1180 μm.

2.1.2. SFE and GAME experiments

2.1.2.1. Set up. Oil extraction by supercritical CO_2 was performed using a device designed by Separex (France). The extractor vessel has a capacity of 2 L, with maximal working pressure, temperature and flow rate of 70 MP, 150 °C and 25 kg CO_2/h, respectively. The experimental setup is presented in Fig. 1. For GAME experiments, a water circuit ensures the mechanical compression of the seed bed, water being used as hydraulic fluid. CO_2 (purity 99,5%) was purchased from Air Liquide, France.

2.1.2.2. SFE experimental procedure. The extractor vessel was first pre-heated at the desired temperature (for 45 to 60 min). Grounded grape seeds (200.0 ± 0.1 g) were introduced into the extractor vessel, above a PET filter (0,45 μm, Sefar-Fyltis, France) to prevent the seed particles to clog the CO_2 circulation lines. The recirculation valve and all exit valves were then closed and the extractor was pressurized at the desired pressure using the CO_2 pump. The CO_2 pressure was manually maintained in the extractor by a back-pressure valve (BRP 1, Fig. 1) located between the extractor and the cyclonic separators. When the desired pressure was reached, the recirculation valve was opened and extraction was maintained for 120 min, under a continuous flux of CO_2. Pressure in the separators was not controlled during the experiments and was comprised between 4.5 and 5.6 MPa. Temperatures were maintained at 60 ± 1 °C and 35 ± 1 °C, respectively for separators 1 and 2. The extracts were collected during the experiments from the two separators.

2.1.2.3. GAME experimental procedure. The pressing chamber was pre-heated between 45 and 60 min before the experiment. Grounded seeds (200.0 ± 0.1 g) were inserted

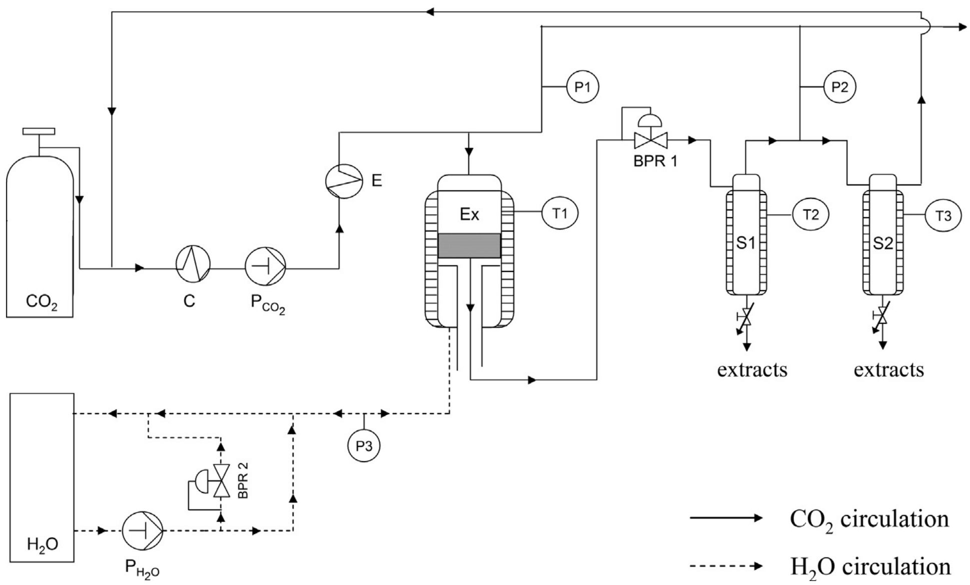

Fig. 1. Experimental setup for SFE and GAME. BPR: back-pressure regulator valve, C: condenser, E: heat exchanger, Ex: extractor, S1/S2: separator 1 and 2, P: pressure sensor, P_{CO2}/P_{H2O}: CO_2/H_2O pump, T: temperature sensor.

into the chamber, above a PET filter (0.45 μm, Sefar-Fyltis, France) allowing the separation between the seeds and the piston pressing surface. The piston was raised in the pressing chamber (extractor), so that the distance between the top of the seed bed and the top of the extractor reached 5.0 ± 0.2 cm. Seeds were equilibrated at the pressing chamber temperature for 30 min. After seed preheating, the CO_2 pump was started to increase CO_2 pressure in the pressing chamber. The CO_2 pressure was adjusted to the desired pressure by the back-pressure valve (BPR 1, Fig. 1). During this operation, the piston position was maintained at its initial level in the chamber. Once CO_2 pressure was reached, the piston was raised for seeds bed compaction. The desired absolute mechanical pressure was then manually adjusted using the back-pressure valve BPR 2 (Fig. 1).

In the case of the GAME experiment, the effective mechanical pressure applied on the seeds is defined by the absolute mechanical pressure (P3, Fig. 1) minus the CO_2 pressure in the pressing chamber (P1, Fig. 1) [22].

For SFE and GAME, separators were cleaned with petroleum ether after each experiment and the recovered fraction was pooled to the last collected extract.

2.1.3. Cold pressing experiment

Oil expression was carried out on a Komet screw press (S87G model, IBG Monforts, Germany). An R6 screw was used for all the experiments. Screw pressing parameters were set at a screw rotation speed of 40 rpm, with a die diameter of 15 mm. Oil temperature was measured (1 °C precision) using a type K thermocouple placed in one perforation of the screw barrel close to the screw head. Before screw pressing experiments, the press head was pre-heated at 90 °C (1 °C precision) for 20 min using a temperature-regulated heating ring. Seeds were fed (throughout a hopper) in the press on demand, by gravity.

2.2. Analytical procedures

2.2.1. Chemicals

Analytical grade *n*-hexane, ethanol, methanol, Na_2CO_3, and Folin–Ciocatleu reagent were purchased from VWR, France. Water was obtained from the milli-Q water purification system (Millipore Corporation, USA). Gallic acid (Sigma-Aldrich, France) served as a standard for the quantification of total polyphenol.

2.2.2. Extracts preparation

Extracts were centrifuged before analysis. For SFE and GAME, extracts were centrifuged for 20 min, 5000 rpm at room temperature. Screw pressing crude oils were centrifuged (10 min, 3000 *g*, room temperature) to separate oil from sediments. Clarified oil was stored at – 20 °C until assessment of total polyphenol content. Oil extraction yield was defined as the ratio of the mass of clarified oil recovered from extraction to the mass of oil originally present in the seeds for screw pressing experiments, and as the ratio of the mass of the oil phase recovered from SFE or GAME experiments to the mass of oil originally present in the seeds.

2.2.3. Extraction and quantification of phenolic compounds

For total polyphenol content of seeds, extraction was performed according to the procedure described by Boussetta et al. (2012) [25].

Extraction of phenolic compounds from oil was done according to the method of Maier et al. (2009) [2] with some modifications. 0.2 g of Tween 20 was added to 5 g of oil sample. After magnetic agitation with 10 mL of a methanol/water solution (80:20; v/v) during 5 min, the mixture was sonicated using an ultrasonic bath for 15 min and mixed again for 5 min. After centrifugation (20 min, 3000 *g*, ambient temperature), the methanolic phase was removed and polyphenol in the oil phase was re-extracted according to the same procedure. After two extractions, the supernatants were pooled, sealed and kept in the dark at 4 °C prior to the polyphenol assay.

Total phenolic compounds in the extracts were quantified according to a method adapted by Boussetta et al. (2012) [25]. Gallic acid diluted either in an ethanolic or methanolic solvent (according to the extraction solvent) was used as a standard solution for preparing the calibration curve ranging from 0 to 80 mg/L (r^2 = 0.994 and r^2 = 0.998, respectively). Results are expressed as grams of Gallic Acid Equivalent (GAE) per 100 g of seed or by mg GAE per kg of oil. Polyphenol quantification was performed in triplicate.

In order to compare in a qualitative manner the polyphenols profiles of the oils, the extracts were also analyzed by HPLC. The apparatus used was an HPLC (Ultimate 3000 LC Packing, Dionex, France) equipped with an automatic injector and a diode array detector. Acquisition and analysis of data were performed using software Chromeleon (Thermo Fisher Scientific, France). The sample (20 μl injected) was eluted through a C18 reverse phase column (Hypersil Gold, 150 × 4,6 mm, 5 μm, Thermo Fisher Scientific, France), which was maintained at a constant temperature of 35 °C. Separation of polyphenols was made by a binary solvent (A and B), whose flow was maintained at 1 ml/min. Solvent A was composed of milli-Q water and acetic acid (99.8, 0.2, v/v), and solvent B was acetonitrile (100%). The elution gradient was based on a method developed by Boussetta (2010) [26] with some modifications (percentage of solvent B are indicated): 0 to 20 min: 6 to 18%, 20 to 35 min: 18 to 28%, 35 to 45 min: 28 to 60%, 45 to 46 min: 60 to 90%, 46 to 50%: isocratic at 90%, 50 to 55 min: 90 to 6%. Polyphenols were detected at a wavelength of 280 nm.

3. Results and discussion

3.1. Supercritical extraction (SFE)

A typical extraction kinetic is presented in Fig. 3 (CO_2 pressure: 53.8 MPa, CO_2 temperature: 104 °C, CO_2 flow rate: 17 kg/h). Crude extracts obtained by SFE were composed of three phases: an oily phase, an aqueous phase, and a third "pasty" phase located at the interface of the two previous phases (Fig. 2). The kinetics presented in Fig. 3 is based on the total extracted masses.

Overall, extraction yields after 120 min of extraction are comprised between 0.061 and 0.067 g extract/g dry seeds. This range of yield, although low, is consistent with another study reporting extraction yield in the range of 0.03 to 0.10 g/g [11]. From results presented in Fig. 3, it can be observed a high variability over the first part of the extraction, where the difficulty of extract recovery is illustrated. The presence of a pasty phase in the extracts increased its viscosity, which could explain this difficulty. Nevertheless, the final global extraction yield is poorly impacted. The pasty phase of the extract was supposed to be composed of waxy compounds (of high molecular weight) co-extracted with oil and water [27]. Co-extraction of a high molecular weight fraction could be favoured by the high CO_2 pressure used. It can also be noted that this pasty phase is not mentioned in previous studies related to SFE of grape seed oil [15,16,11].

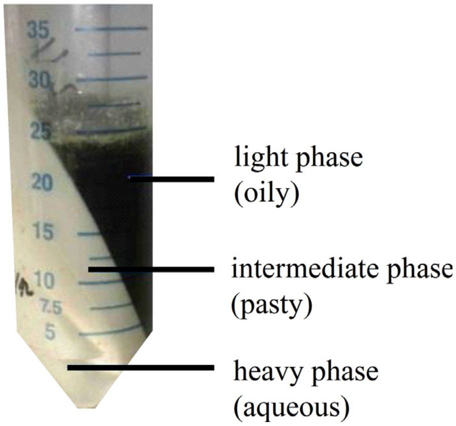

Fig. 2. The three phases obtained after centrifugation of SFE and GAME extracts.

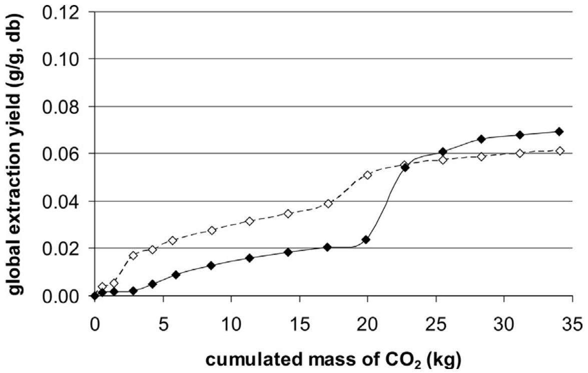

Fig. 3. Typical extraction kinetics (two replicates) for SFE (experimental conditions: CO_2 pressure: 53.8 MPa, CO_2 temperature: 104 °C, CO_2 flow rate: 17 kg/h).

Mass balances considering SFE in the previous experimental conditions were checked (Table 1).

The global and water mass balances are acceptable (less than 7% difference between the input and the output). The oil balance indicates a loss of 17% between the input and the output, which could be explained as follows:

- oil is trapped in the pasty part of the extract and is difficult to collect during extraction and centrifugation, or;
- oil recovery from extracts is not complete.

Table 1
Mass balances of SFE experiments (CO_2 pressure: 53.8 MPa, CO_2 temperature: 104 °C, CO_2 flow rate: 17 kg/h).

	Seeds (g)	Cake (g)	Extract (g)	Balance[a] (%)
Global (g)	200.0 ± 0.1	179.5 ± 2.1	16.8 ± 0.6	1.9 ± 1.4
Water (g)	12.7 ± 0.1	11.0 ± 0.1	2.6 ± 0.4	−6.4 ± 2.2
Oil (g)	24.4 ± 0.1	16.0 ± 0.7	4.2 ± 0.4	17 ± 2

[a] The balance is expressed as the difference between the process input and output, divided by the input.

Table 2
Effect of processing parameters: CO_2 pressure, temperature and flow rate on oil yield and composition of extracts.

CO_2 experimental conditions			Global extract composition			Oil phase characterization	
Pressure (MPa)	Temperature (°C)	Flow rate (kg/h)	Oil phase (%, g/g)	Aqueous phase (%, g/g)	Pasty phase (%, g/g)	Oil yield (%, g/g)	Total polyphenol content in oil (mg EAG/kg oil)
23.0	104	17	11	25	64	6.1	245 ± 61
35.0			30	23	47	11.1	192 ± 14
53.8[a]			28 ± 1	23 ± 4	50 ± 2	17.2 ± 1.1	350 ± 50
53.8	75	17	15	27	58	7.5	270 ± 34
	90		20	32	48	13.6	245 ± 61
	104[a]		28 ± 1	23 ± 4	50 ± 2	17.2 ± 1.1	350 ± 50
	120		23	5	72	12.8	192 ± 14
53.8	104	5	21	30	49	5.7	333 ± 34
		9	35	6	59	8.9	333 ± 35
		14	21	5	74	10.4	190 ± 24
		17[a]	28 ± 1	23 ± 4	50 ± 2	17.2 ± 1.1	350 ± 50

[a] Results for the assay presented in Fig. 3.

The effect of the three processing parameters (CO_2 pressure, temperature, and flow rate) on oil yield and extract composition is summarized in Table 2. The composition of the extracts is presented as the mass proportion of each phase present in the extract.

3.1.1. CO_2 pressure effect

An increase of oil yield with increasing pressure is observed (Table 2). This result is in accordance with classical observations in supercritical fluid extraction, where yield increases with increasing CO_2 pressure [28]. Considering mass proportions between the three phases, no impact of pressure is noticed. For the total polyphenol content of oil, the highest total polyphenol content (350 ± 50 mg GAE/kg oil) is obtained at the highest pressure (53.8 MPa). This result is consistent with observations made by Passos et al. [17]. The authors also noticed a positive impact of pressure increase on the antioxidant capacity of extracts.

3.1.2. CO_2 temperature effect

For temperatures below 100 °C, an increase of oil yield (from 7.5 to 17.2%) is noticed when temperature increases (from 75 to 104 °C). Above 104 °C, a decrease of oil yield is observed (12.8% at 120 °C, Table 2). Extraction performed at 120 °C and 53.8 MPa tends to favour the extraction of the pasty phase (70% against 48 to 58% for temperatures comprised between 75 and 104 °C, respectively). This observation could be explained by the fact that high-molecular-weight compounds could be extracted under a combination of high CO_2 pressures and temperatures. Regarding the total polyphenol content in oil, the lowest level (192 ± 14 mg GAE/kg oil) is obtained for oil extracted at 120 °C, which indicates that degradation or low solubilisation of polyphenol could occur at this extraction temperature.

3.1.3. CO_2 flow rate effect

Oil yield increases from 5.7% to 17.2% by increasing the flow rate from 5 to 17 kg CO_2/h. The composition of the extracts depends on the used flow rate. The oil content in the extracts is quite constant (from 21 to 35%). The water content, however, varies much more; it is at its lower level (6 and 5%) for medium flow rates (9 and 14 kg/h). This observation was not explained since flow rate is not a parameter usually influencing the selectivity of supercritical CO_2. The total polyphenol content in oil seems unaffected by the flow rate, although a lower level (192 mg GAE/kg oil) is obtained at 14 kg CO_2/h.

Although the influence of process parameters on the level of polyphenol in oil is not clear, we can notice that 53.8 MPa, 104 °C and 17 kg CO_2/h are the studied conditions that give the highest amount of polyphenol in oil (350 ± 50 mg GAE/kg oil).

3.2. Gas-Assisted Mechanical Expression (GAME)

As for SFE, extracts obtained by the GAME experiments are composed of three phases: oil, aqueous, and a pasty phase. The extraction kinetics for three effective mechanical pressures is presented in Fig. 4, and expressed in terms of a global extraction yield.

The kinetics shape presented in Fig. 4 is more regular than in case of SFE (Fig. 3), which indicates that no difficulty was encountered to collect the extracts. It can also be noticed that 70 to 80% of the extracts are collected rapidly (in 20 min). This result differs from those obtained by SFE, where extracts are obtained steadily during

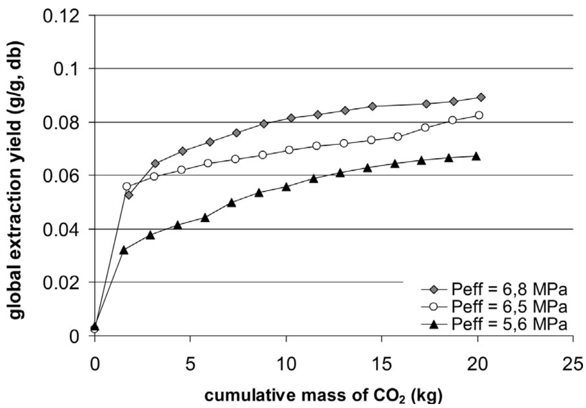

Fig. 4. Effect of the effective mechanical pressure on the extraction kinetics of GAME (experimental conditions: CO_2 pressure: 53 MPa, CO_2 temperature: 104 °C and 17 kg CO_2/h).

Table 3
Effect of effective mechanical pressure on oil yield and composition of extracts for GAME experiments (CO_2 pressure: 53 MPa, CO_2 temperature: 104 °C and CO_2 flow rate: 17 kg/h).

P_{eff} (MPa)	Global extract composition			Oil phase characterization	
	Oil phase (%, g/g)	Aqueous phase (%, g/g)	Pasty phase (%, g/g)	Oil yield (%, g/g)	Total polyphenol content in oil (mg EAG/kg oil)
5.6	46	17	38	29.1	238 ± 8
6.5	36	27	36	34.4	262 ± 6
6.8	45	18	37	43.0	258 ± 7

extraction. If SFE relies on a continuous washing of the extract during extraction [27], the use of a mechanical pressure on the seeds in the GAME experiments leads to an expression of the extracts immediately followed by an entrainment of the latter in the CO_2 flux.

3.2.1. Effective mechanical pressure effect

Final extraction yields are comprised between 0.06 and 0.09 g/g according to the three effective mechanical pressures studied (Fig. 4). It can be noticed that an increase of effective mechanical pressure results in an increase of the extraction yield (Fig. 4). After 70 minutes of GAME (20 kg CO_2), highest global extraction yield (0.09 g/g) is obtained for the highest effective mechanical pressure (6.8 MPa). Oil yield and composition of the extracts are indicated in Table 3.

Composition of the extracts is quite similar for the three effective mechanical pressures studied (Table 3): the oil proportion in the extract is comprised between 36 and 46%, water between 17 and 27% and a pasty content between 36 and 38%. The total polyphenol content in oil is comprised between 238 and 262 mg GAE/kg of oil. The oil yield increases from 29.1 to 43.0% as the effective mechanical pressure increases. During the first minutes of pressing, oil is easily expelled from the seeds. But in order to reach high oil yields, pressure has to be maintained longer, and the higher the pressure, the higher the oil yield, as observed in classical uniaxial expression studies [29,30].

It could then be concluded that although effective mechanical pressure has a key influence on the quantity of extracts, it impacts neither the extract composition nor the polyphenol content in oil.

3.2.2. CO_2 pressure effect

Since CO_2 pressure has an impact on oil yield in SFE, its effect in the GAME process was investigated. The effect of CO_2 pressure on GAME extraction kinetics is shown in Fig. 5.

The extraction kinetics presented in Fig. 5 indicates that CO_2 pressure play an important role in extraction. GAME performed at 5.6 MPa leads to the lowest extraction yield (0.01 g/g). At a pressure of 5.6 MPa and a temperature of 104 °C, CO_2 is in a gaseous state. Therefore, the oil contained in grape seeds cannot be solubilised in CO_2, explaining the low yields. By increasing CO_2 pressure up to 15 MPa, where CO_2 is supercritical, extraction yield increases five-fold. The shape of kinetics is greatly modified when higher CO_2 pressures are applied. At both 45 and 53 MPa, extraction kinetics is similar, which could indicate that beyond 45 MPa, the effect of CO_2 pressure is negligible. Moreover, a plateau is not reached at the end of the experiments, which could indicate that supercritical CO_2 extraction is still effective on the pressed cake. On the contrary, at lower CO_2 pressures, extraction is not effective due to low solubility of supercritical CO_2 towards extractible matter.

Oil yield and composition of extracts are presented in Table 4. Results are compared to a reference trial: a uniaxial expression performed without the use of CO_2.

The reference trial, where seeds were pressed at an equivalent mechanical pressure but without the use of CO_2, does not allow oil extraction (Table 4). This result highlights the beneficial effect of CO_2 pressure on oil extraction.

For experiments performed at a CO_2 pressure below 15 MPa, oil yields are low (around 2%, Table 4). When high CO_2 pressures are used (above 45 MPa), the oil yield reaches a maximum of 35%. These results indicate that solubility of CO_2 towards oil is important in the case of GAME. The oil content in the extract differs according to the CO_2 pressure used. This observation could possibly be explained by the fact that the solubility of CO_2 conditions the composition of extracts. The extract from the experiment performed at 15 MPa contains a high aqueous content (87%, Table 4). It then seems that at 15 MPa and 104 °C, over the three phases, the aqueous one is preferably extracted.

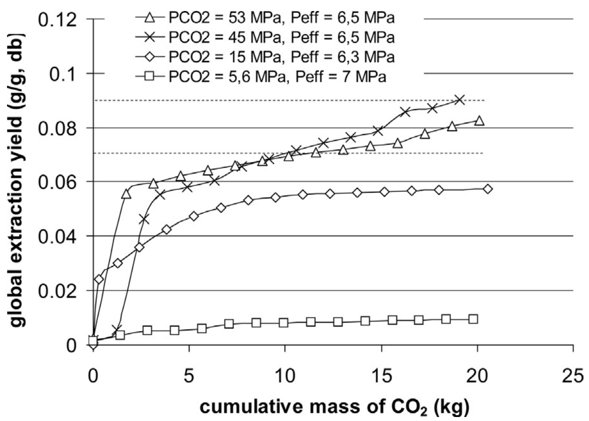

Fig. 5. Effect of CO_2 pressure on extraction yield for GAME (CO_2 temperature: 104 °C, CO_2 flow rate: 17 kg/h). Dotted lines (—) delimits the range of final yield obtained for GAME at a CO_2 pressure of 53 MPa (Fig. 4).

Table 4
Effect of CO_2 pressure on oil yield and composition of extracts obtained by GAME (CO_2 temperature: 104 °C, CO_2 flow rate: 17 kg/h).

Experimental conditions		Global extracts composition			Oil phase characterization	
CO_2 pressure (MPa)	P_{eff} (MPa)	Oil phase (%, g/g)	Aqueous phase (%, g/g)	Pasty phase (%, g/g)	Oil yield (%, g/g)	Total polyphenol content in oil (mg EAG/kg oil)
0 MPa (reference)	7.1	–	–	–	0	–
5.6	7	17	38	46	1.8	201 ± 2
15	6.3	4	87	9	2.1	175 ± 5
45	6.5	35	26	38	34.1	261 ± 10
53	6.5	36	27	36	34.4	262 ± 6

The total polyphenol content in oil also seems to be related to CO_2 pressure. As for oil yield, at low pressures, the polyphenol content in oil is low (175 and 201 mg GAE/kg oil). Its level slightly increases with high pressures (253 and 261 mg GAE/kg oil). It could be supposed that a maximum co-extraction of polyphenol in oil is reached, and that use of pressures above 53 MPa will not improve the amount of co-extracted polyphenol in oil.

3.3. Comparison of supercritical technologies with screw pressing

Maximum oil extraction yield obtained by SFE and GAME are compared to yield obtained by screw pressing in Fig. 6. For each process, the corresponding total polyphenol content in oil is also indicated.

Among the three studied processes, the highest oil yield is obtained by screw pressing (73 ± 5%). This result can be explained by the combination of shear and compression forces applied on the seeds during screw pressing unlike for GAME where expression is only performed by uniaxial compression. For SFE, oil extraction is performed by a continuous flux of CO_2 through the seed bed. The amount of oil extracted then is dependent on the solubility of CO_2 towards oil. Interestingly, uniaxial expression without CO_2 of grape seeds does not allow oil extraction. Given the results presented in Fig. 6, it can be concluded that expression combined with the use of supercritical CO_2 improves oil extraction. This observation was partially explained by Venter et al. [31] and Willems et al. [22]. During expression, part of oil contained in the seeds is expelled, but wets the seed particles. Use of a flux of CO_2 displaces this oil "easily accessible", and therefore increases the oil yield. Considering energy consumption of each one of the selected processes, a specific energy was calculated based on the experimental measure of power consumption (screw pressing) or on data available in the literature (SFE; [32]). SFE energy consumption is related to the mechanical energy needed for fluid compression (388 kWh/t seeds at 750 bar) and to the thermal energy needed for cooling (140.10^4 kJ/t seeds). For screw pressing, the measured specific energy is in the order of 315 kJ/kg seeds, whereas for SFE, it is about 2400 kJ/kg seeds (1000 kJ/kg for mechanical compression of CO_2 and 1400 kJ/kg for cooling according to Eggers [32]). For the GAME process, specific energy can be assumed lower than that for SFE, as the extraction time is lower and the mechanical energy needed for seed compression (about 126 kJ/kg) negligible compared to that for the CO_2 cycle. Then, the GAME process duration of 1.18 h leads to a specific energy of about 1550 kJ/kg considering linear increase of energy consumption with extraction time (2400 kJ/kg for 2 h SFE). Compared to screw pressing, supercritical CO_2 processes need higher specific energy, mainly due to high extraction time and batch processing. Further technical development toward continuous process will increase supercritical CO_2 processes interest, even more since the oil polyphenols content is increased.

For screw pressing, the maximal oil yield attained has an oil polyphenol content of 153 ± 15 mg GAE/kg oil. This result is consistent with other observations [7]. Compared to screw pressing, an increase of the polyphenol concentration in oil is obtained by using supercritical fluid technologies

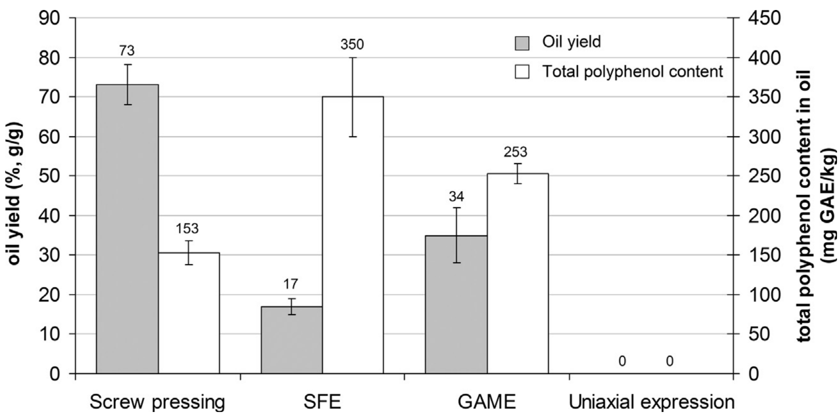

Fig. 6. Comparison between oil yield and total polyphenol content in oils obtained by screw pressing, SFE and GAME.

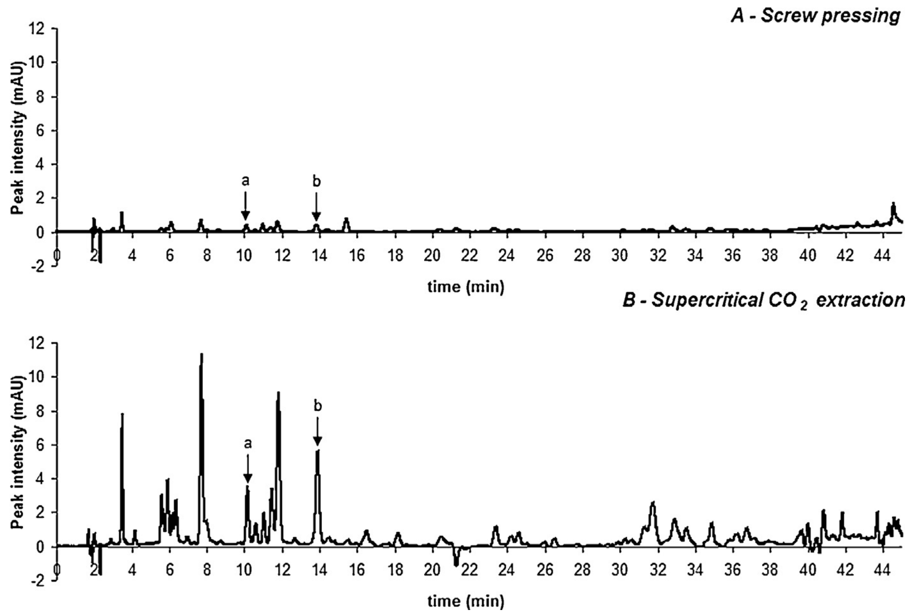

Fig. 7. Polyphenols profiles in oil determined by HPLC, spectrum A: screw pressing oil, spectrum B: oil extracted by supercritical CO_2 (53.8 MPa, 104 °C, 17 kg CO_2/h). Polyphenols identified: a: vanillic acid and b: vanillin.

(Fig. 6). By using SFE, an increase of the oil polyphenol content up to 350 ± 50 mg GAE/kg oil can be achieved. Combining both technologies, oil polyphenol content is increased compared to screw pressing (253 ± 13 mg GAE/kg oil).

As a general observation, a low fraction of the polyphenol initially present in the seeds is extracted in oil (from 0.001% to 0.024%, for a polyphenol content in seeds of 5.7 g/100), indicating a low solubility of polyphenols in oil. However, the use of GAME allows a 2- to 3-fold increase of polyphenol concentration in oil, when compared to oil obtained through classical extraction processes.

In order to assess the qualitative composition of polyphenols in extracted oils, the polyphenols profiles obtained by HLPC are compared in Fig. 7. In this figure are presented polyphenols profiles of oils extracted by screw pressing (spectrum A) and supercritical CO_2 extraction (spectrum B).

To identify the polyphenols in the profiles, polyphenols commonly found in grape seeds were also analyzed as standards: gallic acid, catechin, epicatechin, catechine gallate, epicatechin gallate. None of those polyphenols were identified in the samples (Fig. 7A and B), due to different retention time of the mentioned standards compared to the peaks detected. However, two polyphenols were identified: vanillic acid and vanillin. Our results differ from other authors conclusions regarding polyphenols identification in grape seed oils extracted by supercritical CO_2 [33]; however, the authors used a polar co-solvent, which could explain an enhanced extraction of polyphenols.

It can also be noticed that the polyphenols profiles of the two oils extracted by different processes (Fig. 7) are quite similar, although peaks are more intense in the case of oils extracted by supercritical CO_2. This observation is consistent with results obtained by spectrophotometric assay of total polyphenols indicated in Fig. 6. Comparison of polyphenol profiles then tend to indicate that supercritical CO_2 extraction does not lead to a degradation of polyphenols and could enhance the extraction of other polyphenols.

Extracts obtained by GAME and SFE (using the same supercritical CO_2 conditions: 53.8 MPa, 104 °C and 17 kg/h) were also analyzed using the previously described separation method on a different HPLC apparatus and compared (data not shown). Profiles obtained were identical, therefore suggesting that effective mechanical pressure applied in GAME experiments does not affect the polyphenols profile of oil.

4. Conclusions

In this study, grape seed oil extraction is compared through three processes: screw pressing, supercritical CO_2 extraction, and Gas-Assisted Mechanical Expression. Screw pressing is the most efficient process for producing grape seed oil with a high yield. However, processes using supercritical CO_2 permit an increase of the co-extraction of polyphenol with oil. By combining a uniaxial compression with supercritical CO_2, oil yield is enhanced from 0 (hydraulic pressing, without supercritical CO_2) to 35%, with a higher level of polyphenol in oil than screw pressing. GAME constitutes an interesting alternative process for high-quality oil extraction.

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References

[1] FAOSTAT, Production of crops for 2009. Accessed 23 February 2011, http://faostat.fao.org/.
[2] T. Maier, A. Schieber, D.R. Kammerer, R. Carle, Food Chem. 112 (2009) 551.
[3] N.G. Baydar, G. Özkan, E.S. Cetin, Grasas y Aceites 58 (2007) 29.
[4] C. Crews, P. Hough, J. Godward, P. Brereton, M. Lees, S. Guiet, W. Winkelmann, J. Agric. Food Chem. 54 (2006) 6261.
[5] H. Lutterodt, M. Slavin, M. Whent, E. Turner, L. Yu, Food Chem. 128 (2011) 391.
[6] O. Morin, in: A. Karleskind, J.P. Wolff (Eds.), Oils and fats manual, Intercept, Andover, 1996, p. 143.
[7] S. Bail, G. Stuebiger, S. Krist, H. Unterweger, G. Buchbauer, Food Chem. 108 (2008) 1122.
[8] P. Nikfardjam, Polyphenols in white wines and grape juices and their changes during production, (PhD thesis), Justus von Liebig-Universität, Giessen, Germany, 2001, p. 230.
[9] A. Siger, M. Nogala-Kalucka, E. Lampart-Szczapa, J. Food Lipids 15 (2008) 137.
[10] X. Cao, Y. Ito, J. Chromatogr. A 102 (2003) 117.
[11] A. Molero Gómez, C. Pereyra López, E. Martinez de la Ossa, Chem. Eng. J. Biochem. Eng. J. 61 (1996) 227.
[12] T.H. Beveridge, B. Girard, T. Kopp, J.C. Drover, J. Agric. Food Chem. 53 (2005) 1799.
[13] M. Bourzeix, J.L. Escudier, J. Mourgues, in: C. Flanzy (Ed.), Œnologie : fondements scientifiques et technologiques, Tec&Doc, Lavoisier, Paris, 1998, p. 1144.
[14] L. Johnson, in: C. Akoh, D. Min (Eds.), Food Lipids, second ed., Marcel Dekker, New York, 2002, p. 206.
[15] S. Özkal, J. Am. Chem. Soc. 86 (2009) 1129.
[16] O. Boutin, A. De Nadaï, A.G. Perez, J.H. Ferrasse, M. Beltran, E. Badens, Chem. Eng. Res. Des. 89 (2011) 2477.
[17] C.P. Passos, R.M. Silva, F.A. Da Silva, M.A. Coimbra, C.M. Silva, Chem. Eng. J. 160 (2010) 634.
[18] L. Fiori, J. Supercrit. Fluids 43 (2007) 43.
[19] A.D. Dijkstra, J. Segers, in: J.L. Harwood, F.D. Gunstone, A.D. Dijkstra (Eds.), The Lipid Handbook with CD-ROM, third ed., CRC Press, Boca Raton, FL, 2007, p. 143.
[20] P. Willems, Gas-assisted mechanical expression of oilseeds, (PhD thesis), University of Twente, Twente, The Netherlands, 2007, p. 104.
[21] S. Voges, R. Eggers, A. Pietsch, Sep. Purif. Technol. 63 (2008) 1.
[22] P. Willems, N.J.M. Kuipers, A.B. de Haan, J. Supercrit. Fluids 45 (2008) 298.
[23] Afnor NF E N ISO 665, 2010.
[24] Afnor NF E N ISO 659, 2009.
[25] N. Boussetta, E. Vorobiev, L.H. Le, A. Cordin-Falcimaigne, J.L. Lanoisellé, Food Sci. Technol. 46 (2012) 127.
[26] N. Boussetta, Intensification de l'extraction des polyphénols par électrotechnologies pour la valorisation des marcs de champagne, (PhD thesis), Université de technologie de Compiègne, France, 2010, p. 255.
[27] E. Reverchon, J. Supercrit. Fluids 10 (1997) 1.
[28] G. Brunner, J. Food Eng. 67 (2005) 21.
[29] J.L. Lanoisellé, J.M. Bouvier, Rev. Fr. Corps Gras 41 (1994) 61.
[30] P. Willems, N.J.M. Kuipers, A.B. de Haan, J. Food Eng. 89 (2008) 8.
[31] M. Venter, P. Willems, N. Kuipers, A.B. de Haan, J. Supercrit. Fluids 37 (2006).
[32] R. Eggers, in: J.W. King, G.R. List (Eds.), Supercritical Fluid Technology in Oil and Lipid Chemistry, AOCS Press, Champaign, 1996, p. 435.
[33] M. Palma, L.T. Taylor, J. Chromatogr. A 849 (1999) 117.