



# Policosanols from grape marc: A new step towards a sustainable biorefinery for the wine industry by SC-CO<sub>2</sub> extraction

Carla Da Porto<sup>\*</sup>, Andrea Natolino

University of Udine, Department of Agrifood, Environmental and Animal Sciences, Udine 33100, Italy

## ARTICLE INFO

### Keywords:

Policosanol  
Aliphatic alcohols  
Grape marc  
Mathematical modeling  
SFE  
Biorefinery

## ABSTRACT

Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction of policosanol (PC) from grape marc was investigated for the first time. Employing the broken plus intact cells (BIC) model (Sovová's model) the investigation focused on analyzing the SC-CO<sub>2</sub> process to extract the nonpolar fraction from grape marc efficiently. Operating conditions for SC-CO<sub>2</sub> extraction—280 bar pressure, 70 °C temperature, and a flow rate of 10 kgCO<sub>2</sub>/h—yielded the highest policosanol content. The extracted policosanol ranged between 3922 and 4083 mg/kg<sub>DM</sub>, constituting approximately 8 % of the total extraction yield. Surprisingly, this amount of PC was of the same order of magnitude found in beeswax yellow, a well-known rich natural source of PC. The primary aliphatic alcohols found in the PC from grape marc were hexacosanol, octacosanol, and triacontanol. These findings were consistent with grape marc samples from other Italian regions. Furthermore, a comparative analysis between SC-CO<sub>2</sub> and Soxhlet extraction methods for PC was carried out.

## 1. Introduction

Grape marc, comprising 20–30 % of processed grapes, is a substantial by-product of the wine sector [3]. Global wine production in 2021 yielded around 260 million hectoliters, generating over 6 million tons of grape marc in 2022 [30]. The composition of grape marc, influenced by various factors, primarily includes skins, seeds, and stems, with skins constituting almost half of its weight [21]. Despite its environmental and economic implications due to pollution and seasonality, grape marc remains an undervalued resource rich in polyphenols, lipids, proteins, dietary fiber, cellulose, hemicellulose, and lignin [35].

Researchers have predominantly explored grape marc as a source for polyphenol extraction, given its potential application as valuable biomolecules in biorefinery contexts [4,12,17,23,24,36,45]. However, knowledge gaps exist regarding the extraction of nonpolar compounds like waxes from grape marc. The wax coating in grape skins and seeds could be of particular interest due to its composition and content in policosanol, a group of long-chain aliphatic alcohols (C<sub>20</sub>–C<sub>36</sub>) [28,45,46].

Although originally extracted from sugarcane wax or beeswax [20], policosanol can also be obtained from a wide range of natural sources as reviewed in Table 1. Policosanol is widely used in the fields of dietary food supplements, food additives, cosmetics, pharmaceuticals, and

animal feed additives [11,33]. Olatunji et al. [31] recently reported policosanol effectiveness in treating metabolic syndromes such as dyslipidemia, diabetes, hypertension, and obesity. The most prevalent aliphatic alcohols in policosanol are hexacosanol (C<sub>26</sub>), octacosanol (C<sub>28</sub>), and triacontanol (C<sub>30</sub>) [31].

Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction is an environmentally friendly method employed for policosanol compounds from various natural sources, as reviewed in Table 2. The advantages of SC-CO<sub>2</sub> extraction include its selectivity, relatively mild operating conditions, which minimize the degradation of sensitive compounds, and the absence of potentially harmful organic solvents [13].

However, to the best of our knowledge, no published data is available on the extraction of policosanol from grape marc using SC-CO<sub>2</sub>. Hence, employing Sovová's mathematical model, based on the Broken-and-Intact Cell (BIC) concept, considered very robust and rich in its phenomenological interpretation of supercritical extraction, especially when the solid particles are submitted to mechanical grinding pretreatment, this investigation focuses on analyzing the SC-CO<sub>2</sub> process to extract the nonpolar fraction from grape marc efficiently, and on the policosanol content and composition in SC-CO<sub>2</sub> extracts, using a pilot-scale SC-CO<sub>2</sub> plant.

<sup>\*</sup> Correspondence to: Via Sondrio 2/A, Udine 33100, Italy.

E-mail address: [carla.daporto@uniud.it](mailto:carla.daporto@uniud.it) (C. Da Porto).

<https://doi.org/10.1016/j.jcou.2024.102762>

Received 8 December 2023; Received in revised form 14 February 2024; Accepted 8 April 2024

Available online 13 April 2024

2212-9820/© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Nomenclature		t	Extraction time (min)
$a_0$	Specific surface area per unit volume of extraction bed ( $m^{-1}$ )	$t_{CER}$	Extraction time at the end of CER period (min)
$a_s$	Specific area between intact and broken cells ( $m^{-1}$ )	$t_{FER}$	Extraction time at the end of FER period (min)
$c_u$	Solute content in the untreated solid (kg(solute) kg(solid) <sup>-1</sup> )	$x_i$	Concentration in broken cells (kg(solute) kg (insoluble solid) <sup>-1</sup> )
$d_p$	Particle diameter (m)	$x_u$	Concentration in the untreated solid (kg(solute) kg (insoluble solid) <sup>-1</sup> )
$e$	Extraction yield (kg(extract) kg(insoluble solid) <sup>-1</sup> )	$y$	Fluid phase concentration (kg(solute) kg (solvent) <sup>-1</sup> )
$k_f$	Fluid-phase mass transfer coefficient ( $s^{-1}$ )	$y_0$	Initial fluid-phase concentration (kg(solute) kg (solvent) <sup>-1</sup> )
$k_s$	Solid-phase mass transfer coefficient ( $s^{-1}$ )	$y_s$	Solubility (kg(solute) kg (solvent) <sup>-1</sup> )
$N$	Solid load in the extractor (kg)	<i>Greek letters</i>	
$N_m$	Load of insoluble solid (kg)	$\gamma$	Solvent to matrix ratio in the bed (kg(solvent) kg(insoluble solid) <sup>-1</sup> )
$q$	Relative amount of the passed solvent (kg(solvent) kg (insoluble solid) <sup>-1</sup> )	$\rho_a$	Solid apparent density ( $kg/m^3$ )
$q_m$	Relative amount of passed solvent at the end of CER period (kg(solvent) kg(insoluble solid) <sup>-1</sup> )	$\rho_f$	Solvent density ( $kg/m^3$ )
$q_n$	Relative amount of passed solvent at the end of FER period (kg(solvent) kg(insoluble solid) <sup>-1</sup> )	$\rho_s$	Solid real density ( $kg/m^3$ )
$\dot{Q}$	Solvent flow rate (kg/s)	$\theta_e$	Dimensionless external mass transfer resistance
$r$	Grinding efficiency (fraction of broken cells)	$\theta_i$	Dimensionless internal mass transfer resistance
		$\epsilon$	Bed void fraction

**Table 1**  
Total policosanol content and prevalent aliphatic alcohols (AAs) of various natural sources.

Natural sources	Total Policosanol	Prevalent aliphatic alcohols in policosanol			Ref.
		Hexacosanol (C26)	Octacosanol (C28)	Triacosanol (C30)	
	(mg/kg)		(mg/kg)		
Beeswax-brown	12,000 <sup>a</sup>	1700	2000	5700	Irmak et al. [20]
Beeswax-yellow	5200 <sup>a</sup>	860	900	2300	Irmak et al. [20]
Sugar cane peel	270 <sup>a</sup>	23	219	16	Irmak et al. [20]
Crude Wheat germ oil-solids	628 <sup>a</sup>	164	127	55	Irmak et al. [20]
Green tea ( <i>Camellia sinensis</i> ) leaves	726.2–1363.6 <sup>d</sup>	36.13–172.65	189.7–459.79	162.59–571.85	Choi et al. [7]
	(mg/ kg of dry weight)		(mg/ kg of dry weight)		
Corn kernel pericarp	72.7–110.9 <sup>f</sup>	3.77–8.53	11.48–14.1	33.63–46.3	Harrabi et al. (2009)
Corn kernel endosperm	4–16.2 <sup>f</sup>	0.95–2.81	0.21–0.84	1.01–6.07	Harrabi et al. (2009)
Corn kernel germ	19.3–37.1 <sup>f</sup>	1.32–1.63	2.15–3.41		Harrabi et al. (2009)
	(mg/kg of oil)		(mg/kg of oil)		
Pomegranate ( <i>Punica granatum</i> ) seed oil	118–185 <sup>b</sup>	4.7–15.9	41–100		Caligiani et al. [5]
Hemp ( <i>Cannabis sativa</i> L.) seed oil	17.78–122.2 <sup>c</sup>	6.25–57.11	1.37–22.36		Blasi et al. [4]
Milk thistle ( <i>Silybum marianum</i> L.) oil	574.49–987.68				Harrabi et al. [17]
Tomato ( <i>Solanum lycopersicum</i> L.) seed oil	64.69–71.88 <sup>e</sup>	15.67–18.08	28.04–28.19		Giuffrè and Capocasale [15]
Canola oil	23.179 <sup>g</sup>	2798	4777	9236	Jung et al. [23]
Corn oil	3.014 <sup>g</sup>	0.358	0.337	0.561	Jung et al. [23]
Grape seed oil	245.153 <sup>g</sup>	84,566	53,958	24,008	Jung et al. [23]
Olive oil	32.013 <sup>g</sup>	12.72	4025	0.438	Jung et al. [23]
Perilla seed oil	427.829 <sup>g</sup>	55.26	239.35	40.68	Jung et al. [23]
Ricebrain oil	171.169 <sup>g</sup>	34,364	33,954	61,034	Jung et al. [23]
Safflower oil	20.420 <sup>g</sup>	4064	5141	3972	Jung et al. [23]
Sunflower oil	37.717 <sup>g</sup>	4.66	7435	9355	Jung et al. [23]
Soybean oil	7.399 <sup>g</sup>	0.55	0.729	2351	Jung et al. [23]
Sesame oil	6.026 <sup>g</sup>	0.26	1936	1177	Jung et al. [23]
	(mg/100 g)		(mg/100 g)		
Seedlings of Oat ( <i>Avena sativa</i> L.)	477.1–647.7 <sup>h</sup>	331.0–559.3	23.1–25.7		Lee et al. [24]
			( $\mu g/g$ of wet biomass)		
Antarctic Krill ( <i>Euphausia superba</i> Dana)			10.6		[14]

<sup>a</sup> Total of 9 aliphatic alcohols (AAs) (C20–C24, C26–C28 and C30).

<sup>b</sup> Total of 9 AAs (C20–C28).

<sup>c</sup> Total of 7 AAs (C22–C28).

<sup>d</sup> Total of 13 AAs (C20–C24, C26–C34).

<sup>e</sup> Total of 7 AAs (C22–C28).

<sup>f</sup> Total of 6 AAs (C22, C24, C26, C28, C30 and C32).

<sup>g</sup> Total of 16 AAs (C20–C36).

<sup>h</sup> Total of 9 AAs (C20–C24, C26–C28 and C30).

## 2. Material and methods

### 2.1. Grape marc material

Grape marc from white grape (*Vitis vinifera* L.) varieties were collected in Friuli Venezia-Giulia region (Italy). Grape marc was dried in an oven at 40 °C for 24–36 h. Moisture content was determined by oven drying to constant weight at 105 °C. Prior the SC-CO<sub>2</sub> extraction the grape marc was milled by a domestic grinder and particles characterized by size classification in a standard sifter with several mesh sizes. Mean particle diameter was determined according to Sauter's equation [36] to a set of fractions within previous mesh sized. The true density ( $\rho_s$ ) of raw material was determined by helium gas pycnometry (Pycnomatic ATC, Thermo electron corporation, Milan, Italy) and the apparent density ( $\rho_a$ ) was calculated by dividing the feed mass by the vessel volume. Physical and chemical characterization of grape marc resulted: moisture 10.96 ± 0.17% w/w; mean particle diameter ( $d_p$ ) 0.663 ± 0.014 mm; true density ( $\rho_s$ ) 1262 ± 19 kg/m<sup>3</sup> and apparent density ( $\rho_a$ ) 293.95 ± 0.17 kg/m<sup>3</sup>.

### 2.2. Chemicals

Carbon dioxide (mass fraction purity 0.999 in the liquid phase was supplied by Sapio S.r.l. (Udine, Italy). The standards used for GC-MS analysis, hexacosanol (C26), octacosanol (C28) and triacontanol (C30) were purchased from Sigma-Aldrich (Milan, Italy). Bis (trimethylsilyl) trifluoro acetamide (BSTFA) from Carlo Erba (Milan, Italy) was used as the derivatization reagent. All other solvents and reagents used in analytical determinations were Sigma-Aldrich Co. (Milan, Italy), pro analysis type.

### 2.3. Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction

The entire experimental process took place in a pilot-scale plant for Supercritical Fluid Extraction (SFE) located at University of Udine, Italy. This plant, produced by Separeco S.r.l. (Pinerolo, Italy) known as SCF100serie 3 PLC-GR-DLMP, was equipped with a cylindrical extraction vessel (E<sub>1</sub>) with a capacity of 1 L (length of 33.9 cm and inner diameter of 6.2 cm), followed by two separators (S<sub>1</sub>, S<sub>2</sub>) each with a capacity of 0.3 L, and a storage tank (B<sub>1</sub>) where CO<sub>2</sub> was stored and recycled (Fig. 1). For the extraction process, 0.3 kg of ground grape marc was introduced into the extractor between two thick layers of glass beads (mean diameter of 0.005 m) placed at the bottom and top of the extractor. During the pre-extraction phase, S<sub>1</sub> (gravimetric separator)

and S<sub>2</sub> (cyclonic separator) were set at pressure of 50 bar. Sampling of extracts was conducted at the outlet of S<sub>1</sub>. The extraction time was fixed at 180 min.

SC-CO<sub>2</sub> extractions were then conducted under various conditions, involving different pressure levels (240, 280, and 320 bar), temperatures (50, 60, and 70 °C), and CO<sub>2</sub> flow rates (5, 7, and 10 kg/h), while maintaining constant the mean particle diameter ( $d_p$ ) at 0.663 ± 0.014 mm. The particle size was kept unchanged to preserve process efficiency, since preliminary tests carried out reducing the particle size and increasing pressure highlighted an increase in internal mass transfer resistance. This resulted in irregular compaction within the extraction bed, forming preferential paths (channeling) and subsequently reducing the effective contact between the fluid and the raw-material matrix. Similar findings were reported by other Authors in related studies [12, 34,37].

At intervals of 30 minutes, samples of SC-CO<sub>2</sub> grape extracts were collected in volumetric flasks and their weights were measured using a precise analytical balance (± 0.0001 g). This allowed for the determination of yields, which were then used for the overall extraction curves (OECs). The extraction yield was expressed as yield (% w/w) = [(weight of the extract)/(weight of the dried sample) × 100]. The experimental data were used to model supercritical CO<sub>2</sub> extraction of lipids from grape marc, with a model based on broken and intact cells, developed by Sovová [42]

### 2.4. Soxhlet extraction

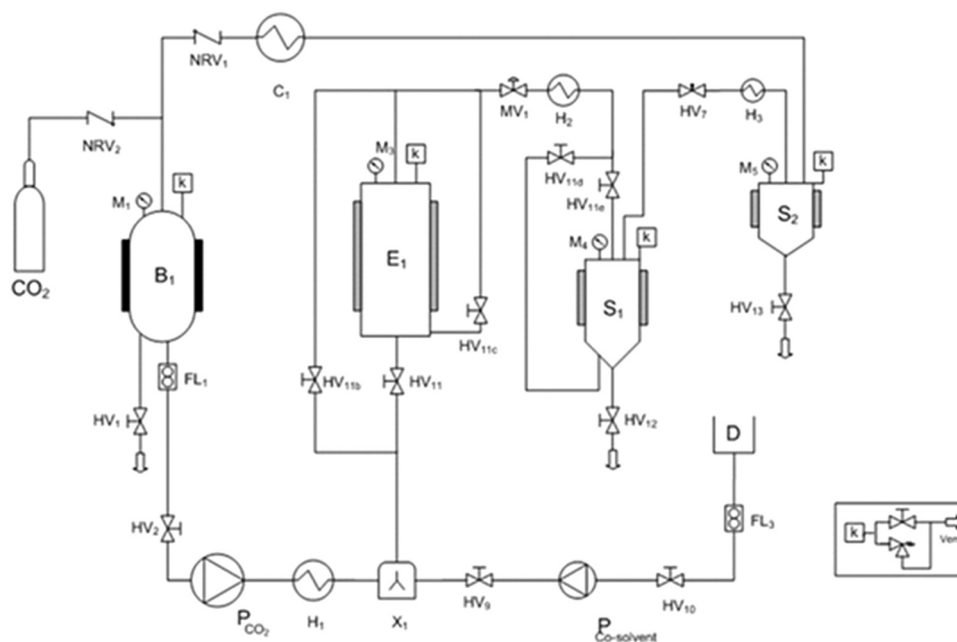
About 15 g of grape marc were transferred into a filter paper extraction thimble and extracted with 200 mL *n*-hexane for 8 h at a maximum temperature of 70 °C in a Soxhlet apparatus. After extraction was completed, *n*-hexane was removed under reduce pressure using a rotary evaporator (Rotavapor R210, Buchi, Flawil, Switzerland). Then, the flask was placed into a desiccator until a constant weight was attained. The extraction was performed in triplicate.

### 2.5. GC-MS analysis of policosanols

The method employed for sample preparation followed the procedure described by Haim et al. [16], with minor adjustments. Initially, 100 µL each of SC-CO<sub>2</sub> and Soxhlet extracts were combined with 100 µL of a betulin solution (used as an internal standard) containing 100 µg/mL in heptane, along with 1 mL of an ethanolic NaOH solution (1 N NaOH dissolved in ethanol/water 80/20 v/v). The mixture was vigorously shaken using a vortex for 30 s and then subjected to saponification for 1 h at 80 °C. To extract policosanols, a liquid-liquid

**Table 2**  
Supercritical CO<sub>2</sub> extraction of policosanols from various natural sources.

Natural sources	Total Policosanols (mg/kg <sub>DM</sub> )	Prevalent aliphatic alcohols in policosanols			Ref.
		Hexacosanol (C26)	Octacosanol (C28)	Triacanthanol (C30)	
		(mg/kg <sub>DM</sub> )			
Sugarcane rind	2208.2	129.1	190.28	142.7	Attard et al. [2]
Sugarcane leaves	613.7	19.6	110.0	90.9	Attard et al. [2]
Sugarcane bagasse	913.6	43.3	716.7	91.5	Attard et al. [2]
Wheat straw wax			1570		[39]
<i>Miscanthus sinensis</i> wax					
Stems	137.2	6.3	26.4	84.3	Attard et al. [1]
Leaves	549.4	16.2	54.2	138.5	Attard et al. [1]
<i>Miscanthus x. Giganteus</i> wax					
Stem	282	12.7	210.2	36.6	Attard et al. [1]
Leaves	793	31.5	227.7	157.8	Attard et al. [1]
		(mg/kg <sub>extract</sub> )			
Quince ( <i>Cydonia oblonga</i> Mill.) wax		129,400	104,400	42,700	Lorenz et al. [27]
Sorghum distiller's grains			16,300–34,000		Wang et al. [43]
Sugarcane juice filter mud			12,970		Ou et al. [32]
Sugarcane bagasse			467		Ou et al. [32]



**Fig. 1.** SFE pilot plant: (B1) storage tank; (E1) extraction vessel; (S1,S2) separators; (H#) heater exchangers; (C1) condenser; (HV#) hand valves; (MV1) membrane valve; (NVR#) no return valves; (P) diaphragm pumps; (F1) flowmeter; (M#) manometers; (k) safety devices; (FL1) Coriolis mass flowmeter; (D) co-solvent storage tank; (X#) mixer.

separation was carried out by adding 2 mL of heptane to the mixture, followed by vortex mixing for 1 min and heating for 10 minutes at 70 °C. Afterward, the organic phase was collected and transferred to a 10 mL test tube. This extraction step was repeated three times, and the organic phases were combined. The solvent was completely evaporated using a nitrogen stream, leaving behind a dry residue. The residue was then reconstituted in 500  $\mu$ L of the derivatizing agent BSTFA for 20 min at 80 °C. Finally, 1  $\mu$ L of the reconstituted sample was injected into the GC-MS system for analysis.

The GC-MS analysis was performed using a GC model GC-2010 (Shimadzu, Kyoto, Japan) equipped with a mass spectrometry detector model GCMS-QP2010. The column used was an HP-5 MS (30 m  $\times$  250  $\mu$ m I.D., 0.25  $\mu$ m film thickness) from Agilent Technologies (California, USA). The oven temperature program was set as follows: an initial temperature of 200 °C, followed by a ramp of 10 °C per minute up to 300 °C, where it was held for 10 minutes. Helium was utilized as the carrier gas at a flow rate of 1 mL/min. The injection was done using a splitless method at 300 °C, and the interface was heated at 300 °C as well. For mass spectrometry, the ion source temperature was set at 230 °C, with an electron energy of 70 eV, and the selected ion monitoring mode was used. A solvent delay of 6 min was implemented. The mass spectrophotometer was configured in the SIM mode within the  $m/z$  ratio range of 40–600. Peak identification was accomplished using software libraries (NIST MS 107 and NIST 21) and by comparing the data with literature references (<https://webbook.nist.gov/chemistry/>), based on the target ions (1-hexacosanol, 439; 1-octacosanol, 467; 1-triacontanol, 495) and their corresponding retention times. To quantify 1-hexacosanol (C26), 1-octacosanol (C28), and 1-triacontanol (C30), calibration curves for each compound were established at various concentrations (ranging from 15 to 300  $\mu$ g/mL). All measurements were performed in triplicate.

## 2.6. Kinetic modeling

The present study employs the broken and intact cells model (BIC) originally proposed by Sovová [41] and its modifications [42] to investigate the SC-CO<sub>2</sub> extraction of nonpolar fraction from grape marc. The methodology for fitting adopted in this study began with an initial

fitting of the experimental data utilizing the simplified model proposed by Sovová [42]. This was followed by a transition to the comprehensive model also proposed by Sovová [42].

The BIC model divides the overall extraction curve (OEC) into three distinct periods: (1) the constant extraction rate (CER) period, characterized by a rapid extraction rate determined by convective solvent film resistance; (2) the falling extraction rate (FER) period, where diffusion mechanism starts to dominate and convection takes place simultaneously; and (3) the diffusion-controlled (DC) period where mass transfer occurs mainly via slow diffusion through the bed and inside the solid substrate particles.

To apply the BIC model, preliminary determination of experimental yield ( $e_{exp}$ ), relative amount of passed solvent ( $q$ ), and solute content in the untreated solid ( $c_u$ ) is essential.

$$e_{exp} = \frac{E}{N_m} \quad (1)$$

$$q = \frac{M}{N_m} \quad (2)$$

Where  $E$  is the amount of extract,  $M$  is the mass of passed solvent. The mass of insoluble solid,  $N_m$  is calculated as:

$$N_m = (1 - c_u) N \quad (3)$$

where  $c_u$  is the solute content in the untreated solid, and  $N$  is the solid loaded in the extractor. The value of  $c_u$  is equal to the asymptotic extraction yield at infinite time. It is calculated by a preliminary fitting of the model equations on experimental data. The solute weight fraction in the untreated solid ( $x_u$ ) can be calculated as follows:

$$x_u = \frac{c_u}{1 - c_u} \quad (4)$$

The bed characteristics, porosity ( $\epsilon$ ), specific surface area per unit volume of extraction bed ( $a_0$ ) and solvent to matrix ratio in the bed ( $\gamma$ ), can be calculated with the following equations:

$$\epsilon = 1 - \frac{\rho_u}{\rho_s} \quad (5)$$

$$a_0 = 6 \frac{1 - \varepsilon}{d} \tag{6}$$

$$\gamma = \frac{\rho_f \varepsilon}{\rho_s (1 - \varepsilon)} \tag{7}$$

The BIC model employs equations to calculate the cumulative mass of extract ( $e$ ) as a function of time ( $t$ ) during each of these three periods [42].

$$\text{CER} : e = qy_s \left[ 1 - \exp\left(-\frac{1}{\theta_e}\right) \right] \text{ for } 0 \leq q < q_m \tag{8}$$

$$\text{FER} : e = qy_s - rx_i \theta_e \exp\left(\frac{\beta}{\theta_e} \ln\left\{ 1 + \frac{1}{r} \left[ \exp\left(\frac{q - q_m}{\theta_i \gamma}\right) - 1 \right] \right\} - \frac{1}{\theta_e}\right) \text{ for } q_m \leq q < q_n \tag{9}$$

$$\text{DC} : e = x_u \left[ 1 - \beta \ln\left\{ 1 + (1 - r) \left[ \exp\left(\frac{1}{\beta}\right) - 1 \right] \exp\left(\frac{q - q_m}{\gamma \theta_i}\right) \right\} \right] \text{ for } q \geq q_n \tag{10}$$

where:

$$\theta_i = \frac{(1 - \varepsilon) \dot{Q}}{\gamma k_s a_s N_m} \tag{11}$$

$$\theta_e = \frac{\varepsilon \dot{Q}}{\gamma k_f a_0 N_m} \tag{12}$$

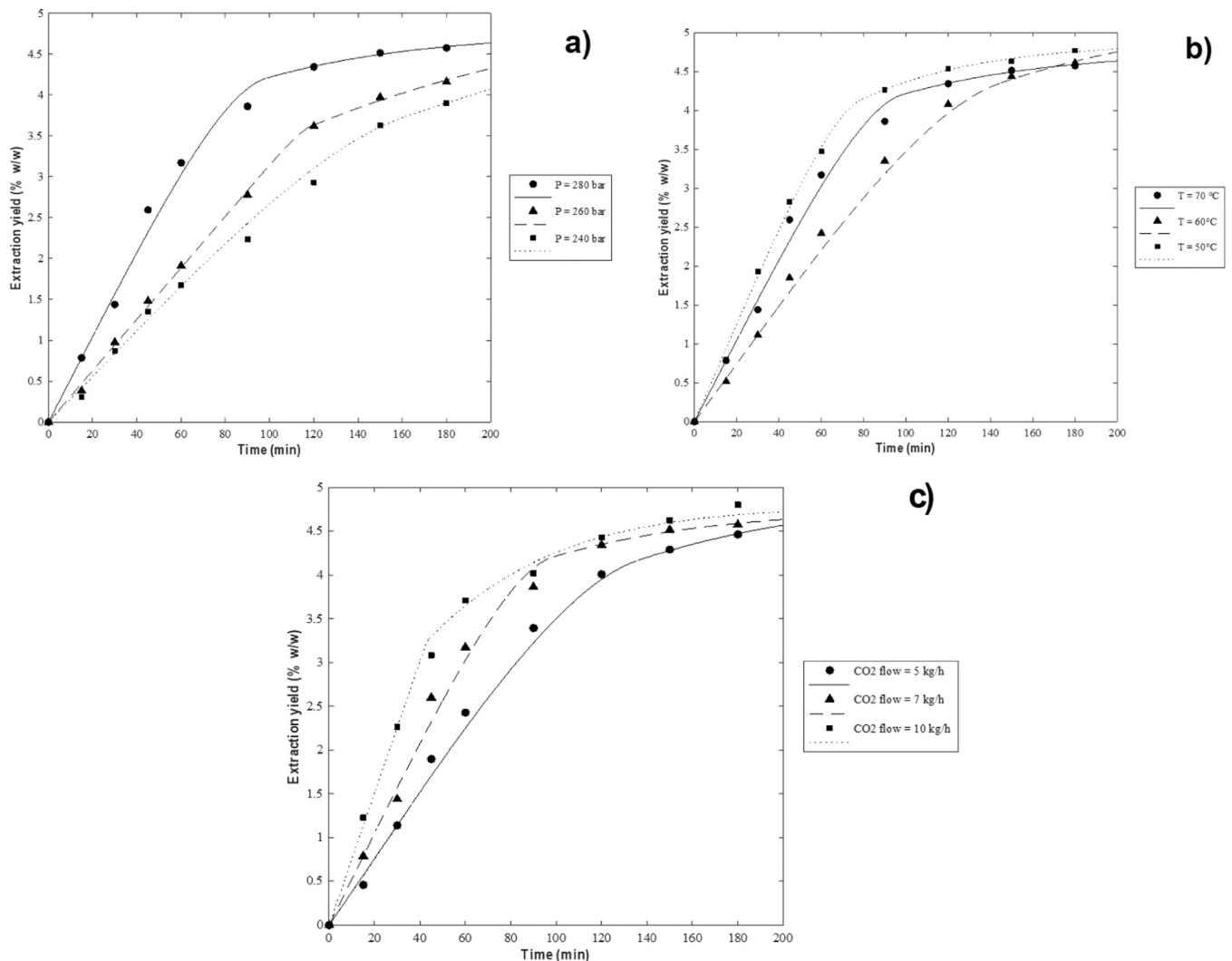
$$q_m = \frac{rx_u \theta_e}{y_s} \tag{13}$$

$$q_n = q_m + \gamma \theta_i \ln\left[ 1 - r + r \exp\left(\frac{1}{\beta}\right) \right] \tag{14}$$

$$\beta = \frac{\gamma \theta_i y_s}{x_u} \tag{15}$$

The adjustable parameters  $r$ ,  $k_s$  and  $k_f$  are estimated by minimizing the sum of least squares between the experimental and calculated values of  $e$ .

The values of model parameters and graph plots were calculated by Matlab R2016b (MathWorks, Inc, USA). The agreement between the experimental and model values were assessed by the absolute average relative deviation (AARD), as reported in following equation:



**Fig. 2.** a. Effect of pressure at 70 °C and 7 kg/h CO<sub>2</sub> flow rate on the extraction yield of nonpolar compounds from grape marc. Experimental (symbols) and modeled (lines) overall extraction curves (OECs). b. Effect of temperature at 280 bar and 7 kg/h CO<sub>2</sub> flow rate on the extraction yield of nonpolar compounds from grape marc. Experimental (symbols) and modeled (lines) overall extraction curves (OECs). c. Effect of CO<sub>2</sub> flow rate at 280 bar and 70 °C on the extraction yield of nonpolar compounds from grape marc. Experimental (symbols) and modeled (lines) overall extraction curves (OECs).

$$AARD (\%) = \frac{100}{n} \sum_{p=1}^n \left| \frac{X_{calc,p} - X_{exp,p}}{X_{exp,p}} \right| \quad (16)$$

where  $n$  is the number of experimental points composing a kinetic curve  $X_{exp,p}$  is the experimental value at point  $p$ , and  $X_{calc,p}$  is the model value at point  $p$ .

### 3. Results and discussion

#### 3.1. Modeling the OECs

The experimental and modeled SC-CO<sub>2</sub> overall extraction curves (OECs) of lipophilic compounds from grape marc at different pressures (240, 260 and 280 bar), temperature (50, 60 and 70 °C) and CO<sub>2</sub> flow rate (5, 7 and 10 kg/h), keeping constant grape marc particle size 0.663 ± 0.014 mm, are presented in Fig. 2(a–c).

##### 3.1.1. Effect of pressure

Fig. 2a depicts the effect of pressure on the extraction process of grape marc, ranging from 240 to 280 bar at 70 °C and 7 kg/h CO<sub>2</sub> flow rate (Exp N° 1, 2 and 3). The curves of the BIC model provided an excellent fit to the experimental data, demonstrating consistent trends. Increasing pressure resulted in a faster extraction rate, owing to the rise in SC-CO<sub>2</sub> density and, thus, its solvation power. The steeper initial slopes of the OEC at higher pressure suggest that compounds solubility increased, leading to enhance significantly ( $p < 0.05$ ) the extraction yield from 3.90 % to 4.57 % (w/w).

Table 3 presents the kinetic parameters of the BIC model evaluated from experimental data acquired from grape marc extraction. The AARD values ranging from 4.83 % to 11.99 % confirm the reliability of the BIC model in describing the extraction curves of lipophilic compounds from grape marc.

External mass transfer coefficients ( $k_f$ ) were found one order of magnitude larger than internal mass transfer coefficients ( $k_s$ ). This resulted because  $k_s$  relies on the properties of the compounds, the permeability of cell walls, and the capacity of SC-CO<sub>2</sub> to penetrate the particles' inner regions. This points to an inadequate access of SC-CO<sub>2</sub> to the particles' interiors and the slow diffusion rates, probably due to the physical structure of grape marc. Consequently, convection offered a more appropriate representation than the diffusional mechanism. These results accord with previous findings on SC-CO<sub>2</sub> extraction of oil from different plant materials [8,18,26,36].

The increase of solubility ( $y_s$ ) from 7.81·10<sup>-4</sup> to 1.44·10<sup>-3</sup>(kg/

kgCO<sub>2</sub>) at constant temperature and increasing pressure indicates the presence of high driving forces in the fluid phase [25]. The decrease of  $t_{CER}$  and  $t_{FER}$  highlights a reduction in the transition time between the fast and slow extraction periods, implying that an increase in pressure accelerates the release of lipophilic compounds from grape marc.

##### 3.1.2. Effect of temperature

Fig. 2b presents the effects of temperature on the yield of extraction (Exp N° 5, 4, and 1) with a CO<sub>2</sub> flow rate of 7 kg/h at pressure of 280 bar within the range of 50–70 °C. The model curves show a consistent trend and fit well with the experimental data. The curves exhibit common shapes with three distinct periods: CER, FER and, DC period.

As reported in Table 3, at 280 bar the extraction curves at 50, 60 and 70 °C are very close during the last period and show a tendency to overlap. Retrograde solubility behavior [13] might be the primary cause of the yield decrease observed when temperature rises from 50 to 70 °C. The solubility ( $y_s$ ) values, confirm the retrograde solubility behavior, with a decrease in values from 1.70 · 10<sup>-3</sup> (kg/kgCO<sub>2</sub>) at 50 °C to 1.44 · 10<sup>-3</sup> (kg/kgCO<sub>2</sub>) at 70 °C (Table 3). These results accord with previous findings on the SC-CO<sub>2</sub> extraction of compounds from various plant materials [29,38,6]. As can be observed in Table 3, the external mass transfer coefficients ( $k_f$ ) are significantly larger than the internal mass transfer coefficients ( $k_s$ ), signifying that convection dominates over the diffusion mechanism. The  $k_f$  values decrease from 4.75 · 10<sup>-5</sup> to 4.46 · 10<sup>-6</sup> (s<sup>-1</sup>) with increasing temperature from 50 to 70 °C. Meanwhile, the extraction periods show that  $t_{CER}$  and  $t_{FER}$  shorten as the temperature decreases, owing to the fast kinetics of SC-CO<sub>2</sub>.

##### 3.1.3. Effect of CO<sub>2</sub> flow rate

As reported by de Melo et al. [10], CO<sub>2</sub> flow rate is an independent variable influencing the axial dispersion, the convective mass transfer coefficient, and the accumulation in the bulk, but not the thermodynamic and transport properties of the solvent.

Fig. 2c shows the effect of CO<sub>2</sub> flow rate (5,7 and 10 kg/h) at 280 bar and 70 °C on the extraction yield, which significantly ( $p < 0.05$ ) increases from 4.46 % to 4.80 % (w/w) although there is no change in SC-CO<sub>2</sub> density (Exp N° 6, 1 and 7) (Table 3). With increasing CO<sub>2</sub> flow rate from 5 to 10 kg/h, steeper slopes of the OECs can be observed. The high flow rate promotes a greater amount of extract. This may be due to the shortening of the CER period, which depletes the extract found at the highest concentration on the particle surface faster. As reported in Table 3, the increase of CO<sub>2</sub> flow rate leads the mass transfer coefficient in the fluid phase ( $k_f$ ) to increase significantly from 2.29 · 10<sup>-6</sup> to 3.07 · 10<sup>-4</sup> (s<sup>-1</sup>), due to enhanced mass transfer by convection in the higher

**Table 3**

Extraction yield of nonpolar compounds and kinetic parameters of the BIC model evaluated from experimental data acquired from grape marc extraction by SC-CO<sub>2</sub>.

Exp. N°	Pressure (bar)	Temperature (°C)	CO <sub>2</sub> flow rate (kg/h)	$\rho_{CO_2}$ (kg/m <sup>3</sup> )	Yield (% w/w)	r	$k_f$ (s <sup>-1</sup> )	$k_s$ (s <sup>-1</sup> )	$\theta_e$	$\theta_i$	$y_s$ (kg/kgCO <sub>2</sub> )	$t_{CER}$ (min)	$t_{FER}$ (min)	AARD (%)
1	280	70	7	769.5	4.57 <sup>bc</sup> ± 0.03	0.8048	4.4651 · 10 <sup>-6</sup>	8.6399 · 10 <sup>-5</sup>	0.3017	12.4065	1.4469 · 10 <sup>-3</sup>	21.29	98.67	4.83
2	260	70	7	748.5	4.16 <sup>d</sup> ± 0.11	0.7423	2.9106 · 10 <sup>-5</sup>	4.7364 · 10 <sup>-5</sup>	0.3770	28.1553	8.3682 · 10 <sup>-4</sup>	31.92	119.59	6.84
3	240	70	7	723.0	3.90 <sup>e</sup> ± 0.07	0.6651	4.4589 · 10 <sup>-6</sup>	3.3291 · 10 <sup>-5</sup>	0.3275	62.9518	7.8114 · 10 <sup>-4</sup>	33.79	161.10	11.99
4	280	60	7	814.2	4.61 <sup>abc</sup> ± 0.04	0.7093	2.9518 · 10 <sup>-6</sup>	5.5987 · 10 <sup>-5</sup>	0.4332	18.3385	1.0977 · 10 <sup>-3</sup>	39.07	144.72	6.51
5	280	50	7	857.5	4.73 <sup>ab</sup> ± 0.08	0.7438	4.7535 · 10 <sup>-5</sup>	1.0184 · 10 <sup>-4</sup>	0.2546	10.6586	1.7040 · 10 <sup>-3</sup>	14.40	79.10	6.77
6	280	70	5	769.5	4.46 <sup>c</sup> ± 0.12	0.7339	2.2956 · 10 <sup>-6</sup>	6.0556 · 10 <sup>-5</sup>	0.4242	15.5274	1.5472 · 10 <sup>-3</sup>	36.40	135.62	10.06
7	280	70	10	769.5	4.80 <sup>a</sup> ± 0.02	0.7301	3.0789 · 10 <sup>-4</sup>	4.0226 · 10 <sup>-5</sup>	0.0189	10.8195	4.6400 · 10 <sup>-4</sup>	0.64	44.01	4.26

Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

\* Mean values with different letters within a column are significantly different, at  $p < 0.05$ .

amount of fresh CO<sub>2</sub> passing through the packed bed. In addition, at higher flows, there is a decrease in the film thickness around the solid particles and consequently a decrease in the internal mass transfer resistance ( $\theta_i$ ) as shown by the increasing values of  $k_s$  from  $6.05 \cdot 10^{-5}$  to  $4.02 \cdot 10^{-5}$  (s<sup>-1</sup>). At 10 kg/h flow rate, equilibrium solubility of extract compounds in the solvent is not achieved at the outlet of the fixed bed. This is confirmed by the lowest value of solubility ( $y_s$ )  $4.64 \cdot 10^{-4}$  (kg/kgCO<sub>2</sub>) obtained at 10 kg/h employing the BIC model (Table 1). Similar findings have been reported by Soh et al. [40] in supercritical fluid extraction of patchouli oil.

### 3.2. Policosanol content and composition under different SC-CO<sub>2</sub> operating conditions

As shown in Table 4, total policosanol content from grape marc ranged from 3922 to 4083 mg/kg<sub>DM</sub> under different SC-CO<sub>2</sub> operating conditions (pressure, temperature, and CO<sub>2</sub> flow rate), representing approximately 8.5 % of global extraction yield. The amount of policosanol is surprising, considering the rich policosanol content of beeswax yellow reported to be 5200 mg/kg [20].

Total policosanol content results significantly increased with respect to pressure, increasing from 240 to 280 bar (Exp. N°1, 2 and 3), as well as for aliphatic alcohols C26, C28, and C30, with C26 being the most prevalent. This suggests that the solubility of these compounds increases as SC-CO<sub>2</sub> density increases with higher pressure. Increasing temperature from 50 to 70 °C significantly impacted the total policosanol concentration (Exp. N° 5, 4 and 1), with content increasing from 3486 to 4030 mg/kg<sub>DM</sub> due to retrograde solubility behavior. While temperature influenced both C28 and C30 content, only C26 is not affected ( $p > 0.05$ ) by temperature increase.

Finally, it is interesting to note that the effect of the low and high and low flow rates analyzed in this study (Exp. N° 6,1 and 7) do not affect significantly ( $p > 0.05$ ) policosanol content. Only C28, shows significantly increase ( $p < 0.05$ ) from 533 to 862 mg/kg<sub>DM</sub> with increasing CO<sub>2</sub> flow rate from 5 to 10 kgCO<sub>2</sub>/h. According to Table 4, 280 bar, 70 °C and 10 kg/h of CO<sub>2</sub> flow rate are the operating conditions that provide a higher extraction of policosanols within the studied range.

As shown in Table 4, modifying the SC-CO<sub>2</sub> operating conditions (pressure, temperature, and CO<sub>2</sub> flow rate) it is possible to obtain different policosanol composition in C26, C28 and C30.

Understanding that wax compound amounts are influenced by grape cultivar, development stages, and environmental conditions [28,45,46], we also investigated the policosanol content and composition of grape marc samples from other regions of Italy (Umbria and Toscana) via SC-CO<sub>2</sub> extraction at 280 bar, 70 °C and 10 kg/h of CO<sub>2</sub> flow rate. As depicted in Table 5, the results of analyzes revealed high policosanol concentration and consistent contents of C26, C28, and C30 in the grape marc samples. It is interesting to note that the percentage of C28 with respect to total policosanol remains almost constant at 35 % in the grape marc samples. These findings further substantiate our feedback.

Ilyas et al. [19] reported that grape marc contains 4–11 % lipids. Given that the yield of nonpolar compounds obtained through SC-CO<sub>2</sub>

**Table 4**  
Policosanol content and composition of grape marc under different SC-CO<sub>2</sub> operating conditions.

Exp.N°	Pressure (bar)	Temperature (°C)	CO <sub>2</sub> flow rate (kg/h)	C26 (mg/kg <sub>DM</sub> )	C28 (mg/kg <sub>DM</sub> )	C30 (mg/kg <sub>DM</sub> )	Total policosanol (mg/kg <sub>DM</sub> )
1	280	70	7	2004 <sup>a*</sup> ± 85	1400 <sup>ab</sup> ± 25	627 <sup>b</sup> ± 25	4030 <sup>a</sup> ± 86
2	260	70	7	1822 <sup>ab</sup> ± 41	1226 <sup>c</sup> ± 20	583 <sup>b</sup> ± 13	3631 <sup>bc</sup> ± 65
3	240	70	7	1644 <sup>b</sup> ± 102	1143 <sup>c</sup> ± 76	532 <sup>b</sup> ± 53	3319 <sup>c</sup> ± 230
4	280	60	7	1801 <sup>ab</sup> ± 57	1254 <sup>bc</sup> ± 15	544 <sup>b</sup> ± 28	3599 <sup>bc</sup> ± 25
5	280	50	7	1998 <sup>a</sup> ± 80	1181 <sup>c</sup> ± 62	306 <sup>c</sup> ± 61	3486 <sup>c</sup> ± 117
6	280	70	5	1994 <sup>a</sup> ± 77	1395 <sup>ab</sup> ± 93	533 <sup>b</sup> ± 45	3922 <sup>ab</sup> ± 183
7	280	70	10	1807 <sup>ab</sup> ± 107	1413 <sup>a</sup> ± 8	862 <sup>a</sup> ± 84	4083 <sup>a</sup> ± 72

Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

\* Mean values with different letters within a column are significantly different, at  $p < 0.05$ .

**Table 5**  
Comparison of policosanol content and composition of grape marc samples originating from different Italian regions.

Grape marc origin	Aliphatic alcohols (mg/kg <sub>DM</sub> )			
	C26	C28	C30	Total Policosanol
Friuli Venezia Giulia	1807 ± 107	1413 ± 8	862 ± 84	4083 ± 72
Umbria	860 ± 95	849 ± 35	720 ± 42	1595 ± 123
Toscana	906 ± 75	927 ± 56	726 ± 67	2559 ± 212

extraction was approximately 4 %, with policosanols constituting around 8 % of this extract, it suggests that grape seed oil may be the most quantitatively significant component of the lipid extract. However, it is evident that additional investigations are warranted to elucidate the composition of the residual extract.

### 3.3. Comparison between SCCO<sub>2</sub> and Soxhlet extraction of policosanol

Policosanol composition of grape marc using sc-CO<sub>2</sub> and Soxhlet with *n*-hexane is given in Table 6.

The results indicate that both techniques extracted similar types of compounds (C26, C28, and C30), but the total policosanol content was smaller in SC-CO<sub>2</sub> extracts than *n*-hexane extracts (4083 ± 72 vs. 4503 ± 60 mg/kg<sub>DM</sub>).

The comparison between SC-CO<sub>2</sub> and Soxhlet extraction demonstrates that supercritical CO<sub>2</sub> can extract approximately the same amount of policosanol extracted with solvent extraction, with the advantages of ensuring a faster and free-solvent process. Higher amounts of 1-hexacosanol have been found in both extracts. Similar results have been obtained by Attard et al. [2] for sugarcane waste.

## 4. Conclusions

The predictions from Sovová's model, demonstrating good agreements with experimental data, offer valuable insights into the mass transfer mechanism governing the extraction process of the nonpolar fraction from grape marc. The amount of extracted policosanol in grape marc from Friuli -Venezia Giulia region was in the range of 4000 mg/

**Table 6**  
Comparison of policosanol composition obtained by SC-CO<sub>2</sub> and Soxhlet extraction from grape marc.

Aliphatic alcohols	SC-CO <sub>2</sub> (280 bar, 70 °C, 10 kgCO <sub>2</sub> /h)	Soxhlet ( <i>n</i> -hexane)
C26	1807 <sup>a*</sup> ± 107	1924 <sup>a</sup> ± 111
C28	1413 <sup>b</sup> ± 8	1609 <sup>a</sup> ± 15
C30	862 <sup>a</sup> ± 84	970 <sup>a</sup> ± 73
Total Policosanol	4083 <sup>b</sup> ± 72	4503 <sup>a</sup> ± 60

Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

\* Mean values with different letters within a row are significantly different, at  $p < 0.05$ .

kgDM, the same order of magnitude of beeswax yellow, a well-known rich natural source of policosanols. The aliphatic alcohols found in the policosanols from grape marc were hexacosanol, octacosanol, and triacontanol. Furthermore, the application of SCCO<sub>2</sub> extraction to grape marc from additional Italian regions (Umbria and Toscana) confirmed the consistently high concentrations of policosanols, along with its distinctive composition of aliphatic alcohols. Considering the yield of nonpolar compounds obtained through SCCO<sub>2</sub> extraction, which was approximately 4 %, and with policosanols constituting around 8 % of this extract, a comprehensive characterization of the composition is essential. This assessment is crucial for determining the SCCO<sub>2</sub> extract's potential direct applicability or the necessity for purification procedures.

The comparison between SC-CO<sub>2</sub> and Soxhlet extraction demonstrates that supercritical CO<sub>2</sub> can extract approximately the same amount of policosanols extracted with solvent extraction, with the advantages of ensuring a faster and free-solvent process.

The surprising high policosanols content found in grape marc highlights its significant implications for the strategic implementation of supercritical fluid extraction in the creation of a sustainable biorefinery framework for wine industry by-products. This finding expands the horizons for valorizing grape marc within the biorefinery context, surpassing the conventional focus on polyphenol extraction, which represents only a fraction of its potential.

The inherent versatility of the supercritical fluid extraction (SFE) system, facilitating the application of successive or sequential extraction conditions, offers the potential for a selective recovery process of both nonpolar and polar fractions from grape marc. Accordingly, it is proposed to initially conduct supercritical carbon dioxide extraction on grape marc for the recovery of policosanols, followed by a subsequent SC-CO<sub>2</sub> extraction with a co-solvent for the extraction of polyphenols, given their limited solubility in SC-CO<sub>2</sub>. The superior quality of SC-CO<sub>2</sub> extracts, surpassing that of other extraction technologies, contributes to their heightened added value. Finally, the raffinate phase of SC-CO<sub>2</sub> extracted grape marc could find utility in other biorefinery processes.

#### CRedit authorship contribution statement

**Andrea Natolino:** Formal analysis. **Carla Da Porto:** Writing – review & editing, Methodology, Conceptualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

#### References

1. T.M. Attard, C.R. McElroy, R.J. Gammons, J.M. Slattery, N. Supanchaiyamat, Supercritical CO<sub>2</sub> extraction as an effective pretreatment step for wax extraction in a *Miscanthus* biorefinery, *Sustain. Chem. Eng.* 4 (2016) 5979–5988, <https://doi.org/10.1021/acscchemeng.6b01220>.
2. T.M. Attard, C.R. McElroy, C.A. Rezende, I. Polikarpov, J.H. Clark, A.J. Hunt, Sugarcane waste as a valuable source of lipophilic molecules, *Ind. Crops Prod.* 76 (2015) 95–103, <https://doi.org/10.1016/j.indcrop.2015.05.077>.
3. C. Beres, G.N. Costa, I. Cabezedo, N.K. da Silva-James, A.S. Teles, A.P. Cruz, S. P. Freitas, Towards integral utilization of grape pomace from winemaking process: a review, *Waste Manag.* 68 (2017) 581–594, <https://doi.org/10.1016/j.wasman.2017.07.017>.
4. F. Blasi, C. Tringaniello, G. Verducci, L. Cossignani, Bioactive minor components of Italian and Extra-European hemp seed oils, *LWT-Food Sci. Technol.* 158 (2022) 113167, <https://doi.org/10.1016/j.lwt.2022.113167>.
5. A. Caligiani, F. Bonzanini, G. Palla, M. Cirilini, R. Bruni, Characterization of a potential nutraceutical ingredient: pomegranate (*Punica granatum* L.) seed oil unsaponifiable fraction, *Plant Food Hum. Nutr.* 65 (2010) 277–283.
6. P.C.K. Cheung, Temperature and pressure effects on supercritical carbon dioxide extraction of n-3 fatty acids from red seaweed, *Food Chem.* 65 (1999) 399–403, [https://doi.org/10.1016/S0308-8146\(98\)00210-6](https://doi.org/10.1016/S0308-8146(98)00210-6).
7. J.S. Choi, S.Y. Park, J.S. Park, S.-K. Park, M.Y. Junk, Contents and compositions of policosanols in green tea (*Camellia sinensis*) leaves, *Food Chem.* 204 (2016) 94–101.
8. T.C. Confortin, I. Toderò, N.I. Canabarro, L. Luft, G.A. Ugalde, J.R.C. Neto, M. A. Mazutti, G.L. Zabot, M.V. Tres, Supercritical CO<sub>2</sub> extraction of compounds from different aerial parts of *Senecio brasiliensis*: mathematical modeling and effects of parameters on extract quality, *J. Supercrit. Fluids* 153 (2019) 104589, <https://doi.org/10.1016/j.supflu.2019.104589>.
9. C. Da Porto, A. Natolino, M. Scalet, Improved sustainability in wine industry byproducts: a scale-up and economical feasibility study for high-value compounds extraction using modified SC-CO<sub>2</sub>, *ACS Omega* (38) (2022) 33845–33857, <https://doi.org/10.1021/acscomega.2c02631>.
10. M.M.R. de Melo, A.J.D. Silvestre, C.M. Silva, Supercritical fluid extraction of vegetable matrices: applications, trends and future perspectives of a convincing green technology, *J. Supercrit. Fluids* 92 (2014) 115–176, <https://doi.org/10.1016/j.supflu.2014.04.007>.
11. N.T. Dunford, J. Edwards, Nutritional bioactive components of wheat straw as affected by genotype and environment, *Bioresour. Technol.* 101 (2010) 422–425.
12. A.M. Farias-Campomanes, M.A. Rostagno, M.A.A. Meireles, Production of polyphenol extracts from grape bagasse using supercritical fluids: yield, extract composition and economic evaluation, *J. Supercrit. Fluids* 77 (2013) 70–78.
13. G. Brunner, *Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes*, Ed. Steinkopff, Darmstadt; Springer, New York, 1994.
14. W. Gao, D. Liu, S. Su, High-performance thin-layer chromatography for quantification of 1-octacosanol in Antarctic krill (*Euphausia superba* Dana), *J. Chromatogr. Sci.* 53 (2015) 811–815.
15. A.M. Giuffrè, M. Capocasale, Policosanols in tomato (*Solanum lycopersicum* L.) seed oil: the effect of cultivar, *J. Oleo Sci.* 64 (2015) 625–631.
16. D. Haim, M. Berrios, A. Valenzuela, L.A. Videla, Trace quantification of 1-octacosanol and 1-triacontanol and their main metabolites in plasma by liquid-liquid extraction coupled with gas chromatography-mass spectrometry, *J. Chromatogr. B* 877 (2009) 4154–4158, <https://doi.org/10.1016/j.jchromb.2009.10.034>.
17. S. Harrabi, A. Ferchichi, A. Bachel, H. Fellah, Policosanols composition, antioxidant and anti-arthritis activities of milk thistle (*Silybum marianum* L.) oil at different seed maturity stages, *Lipids Health Dis.* 17 (1) (2018) 7, <https://doi.org/10.1186/s12944-018-0682-z>.
18. Z. Huang, X. Shi, W. Jiang, Theoretical models for supercritical fluid extraction, *J. Chromatogr. A* 1250 (2012) 2–26, <https://doi.org/10.1016/j.chroma.2012.04.032>.
19. T. Ilyas, P. Chowdhary, D. Chaurasia, E. Gnansounou, A. Pandey, P. Chaturvedi, Sustainable green processing of grape pomace for the production of value-added products: an overview, *Environ. Technol. Innov.* 23 (2021) 101592.
20. S. Irmak, N.T. Dunford, J. Milligan, Policosanols contents of beeswax, sugarcane and wheat extracts, *Food Chem.* 95 (2006) 312–318, <https://doi.org/10.1016/j.foodchem.2005.01.009>.
21. B. Jin, J.M. Kelly, Wine industry residues, in: P.S.N. Nigam, A.A. Pandey (Eds.), *Biotechnology for Agro-Industrial Residues Utilisation: Utilisation of Agro-Residues*, Springer, The Netherlands, 2009, pp. 293–311.
22. Q. Jin, S.F. O'Keefe, A.C. Stewart, A.P. Neilson, Y.T. Kim, H. Huang, Techno-economic analysis of a grape pomace biorefinery: production of seed oil, polyphenols, and biochar, *Food Bioprod. Proc.* 127 (2021) 139–151, <https://doi.org/10.1016/j.fbp.2021.02.002>.
23. D.M. Jung, M.J. Lee, S.H. Suk, M.Y. Jung, A Gas Chromatography-tandem quadrupole mass spectrometric analysis of policosanols in commercial vegetable oils, *J. Food Sci.* 6 (2011) C891–C899.
24. H.-G. Lee, S.-Y. Woo, H.-J. Ahn, J.-Y. Yang, M.-J. Lee, H.-Y. Kim, S.-Y. Song, J.-H. Lee, W.-D. Seo, Comparative analysis of policosanols related to growth times from the seedlings of various Korean oat (*Avena sativa* L.) cultivars and screening for adenosine 50-monophosphate-activated protein kinase (AMPK) activation, *Plants* 11 (2022) 1844, <https://doi.org/10.3390/plants11141844>.
25. K.K. Liang, P.A. Wells, N.R. Foster, Diffusion of fatty acid esters in supercritical carbon dioxide, *Ind. Eng. Chem. Res.* 31 (1992) 390–399, <https://doi.org/10.1021/ie00001a053>.
26. A. López-Padilla, A. Ruiz-Rodríguez, G. Reglero, T. Fornari, Supercritical carbon dioxide extraction of *Calendula officinalis*: kinetic modeling and scaling up study, *J. Supercrit. Fluids* 130 (2017) 292–300, <https://doi.org/10.1016/j.supflu.2017.03.033>.
27. P. Lorenz, M. Berger, J. Bertrams, K. Wende, K. Wenzel, Natural wax constituents of a supercritical fluid CO<sub>2</sub> extract from quince (*Cydonia oblonga* Mill.) pomace, *Anal. Bioanal. Chem.* 391 (2008) 633–646, <https://doi.org/10.1007/s00216-008-2000-5>.
28. J.A.S. Mendes, S.O. Prozil, D.V. Evtuguina, L.P.C. Lopes, Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomaces of variety Touriga Nacional, *Ind. Crops Prod.* 43 (2013) 25–32, <https://doi.org/10.1016/j.indcrop.2012.06.047>.
29. A. Mouahid, I. Bombarda, M. Claeys-Bruno, S.T. Ama, E. Myotte, J.P. Nisteron, C. Crampon, E. Badens, Supercritical CO<sub>2</sub> extraction of Moroccan argan (*Argania spinosa* L.) oil: extraction kinetics and solubility determination, *J. CO<sub>2</sub> Util.* 46 (2021) 101458, <https://doi.org/10.1016/j.jcou.2021.101458>.
30. OIV - International Organisation of Vine and Wine Intergovernmental Organisation, State of the world vine and wine sector 2021, 2022. (<https://www.oiv.int>).



- [31] L.W. Olatunji, A.O. Jimoh, T.U. Muhammad, M.U. Imam, A review of the effects of policosanol on metabolic syndrome, *CCMP* 2 (2022) 100058, <https://doi.org/10.1016/j.ccmp.2022.100058>.
- [32] S. Ou, J. Zhao, Y. Wang, Y. Tian, J. Wang, Preparation of octacosanol from filter mud produced after sugarcane juice clarification, *LWT-Food Sci.* 45 (2012) 295–298, <https://doi.org/10.1016/j.lwt.2011.08.011>.
- [33] I. Pasha, F. Saeed, K. Waqas, F.M. Anjum, M.U. Arshad, Nutraceutical and functional scenario of wheat straw, *Crit Rev Food Sci Nutr* 53 (2013) 287–295.
- [34] C.G. Pereira, A.A.M. Meireles, Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives, *Food Bioprocess Technol.* 3 (2010) 340–372, <https://doi.org/10.1007/s11947-009-0263-2>.
- [35] M. Perra, G. Bacchetta, A. Muntoni, G. De Gioannis, I. Castangi, H.N. Rajha, M. L. Manca, M. Manconi, An outlook on modern and sustainable approaches to the management of grape pomace by integrating green processes, biotechnologies and advanced biomedical approaches, *J. Funct. Foods* 98 (2022) 105276, <https://doi.org/10.1016/j.jff.2022.105276>.
- [36] N.P. Povh, M.O.M. Marques, M.A.A. Meireles, Supercritical CO<sub>2</sub> extraction of essential oil and oleoresin from chamomile (*Chamomilla recutita* [L.] Rauschert), *J. Supercrit. Fluids* 21 (2001) 245–248, [https://doi.org/10.1016/S0896-8446\(01\)00096-1](https://doi.org/10.1016/S0896-8446(01)00096-1).
- [37] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of natural matter, *J. Supercrit. Fluids* 38 (2006) 146–166, <https://doi.org/10.1016/j.supflu.2006.03.020>.
- [38] R.F. Rodrigues, A.T. Tashima, R.M.S. Pereira, R.S. Mohamed, F.A. Cabral, Coumarin solubility and extraction from emburana (*Torresea cearensis*) seeds with supercritical carbon dioxide, *J. Supercrit. Fluids* 43 (2008) 375–382, <https://doi.org/10.1016/j.supflu.2007.07.014>.
- [39] E.H.K. Sin, R. Marriott, A.J. Hunt, J.H. Clark, Identification, quantification, and Chrastil modelling of wheat straw wax extraction using supercritical carbon dioxide, *C. R. Chim.* 17 (2014) 293–300, <https://doi.org/10.1016/j.crci.2013.12.001>.
- [40] S.H. Soh, S. Agarwal, A. Jain, L.Y. Lee, S.K. Chin, S. Jayaraman, Mathematical modeling of mass transfer in supercritical fluid extraction of patchouli oil, *Eng. Rep.* 1 (2019) e12051, <https://doi.org/10.1002/eng2.12051>.
- [41] H. Sovová, Rate of the vegetable oil extraction with supercritical CO<sub>2</sub>-I. Modelling of extraction curves, *Chem. Eng. Sci.* 49 (1994) 409–414, [https://doi.org/10.1016/0009-2509\(94\)87012-8](https://doi.org/10.1016/0009-2509(94)87012-8).
- [42] H. Sovová, Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation, *J. Supercrit. Fluids* 33 (2005) 35–52, <https://doi.org/10.1016/j.supflu.2004.03.005>.
- [43] L. Wang, C.L. Weller, V.L. Schlegel, T.P. Carr, S.R. Cuppett, Comparison of supercritical CO<sub>2</sub> and hexane extraction of lipids from sorghum distillers' grains, *Eur. J. Lipid Sci. Technol.* 109 (2007) 567–574, <https://doi.org/10.1002/ejlt.200700018>.
- [44] P.R. Yaashika, P. Senthil Kumar, S. Varjani, Valorization of agro-industrial wastes for biorefinery process and circular bioeconomy: a critical review, *Bioresour. Technol.* 343 (2022), <https://doi.org/10.1016/j.biortech.2021.126126>.
- [45] M. Yang, Z. Luo, S. Gao, T. Belwal, L. Wang, M. Qi, Z. Ban, B. Wu, F. Wang, L. Li, The chemical composition and potential role of epicuticular and intracuticular wax in four cultivars of table grapes, *Postharvest Biol. Technol.* 173 (2021) 111430, <https://doi.org/10.1016/j.postharvbio.2020.111430>.
- [46] M. Zhang, P. Zhang, S. Lu, Q. Ou-yang, Y. Zhu-ge, R. Tian, H. Jia, J. Fang, Comparative analysis of cuticular wax in various grape cultivars during berry development and after storage, *Front. Nutr.* 8 (2021) 817796, <https://doi.org/10.3389/fnut.2021.817796>.