Fractionation of vanilla oleoresin by supercritical CO_2 technology

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ABSTRACT

In this work it was determined experimentally the vanilla oleoresin solubility in supercritical CO_2 at moderated pressures and it was also studied the vanilla CO_2 extraction/fractionation process. The initial equilibrium concentration of vanilla oleoresin in the CO_2 extract phase was determined by a dynamic method at 140 bar and 180 bar in a range of temperature of 310.5–348.3 K. A significant change in vanilla oleoresin solubility (2–10 g oleoresin/kg CO_2) and selectivity toward the extraction of vanillin (main flavor component) was observed according to the operating temperature and pressure. It was also analyzed the CO_2 batch fractionation of vanilla oleoresin under equilibrium conditions at 310.5 K and 140 bar in order to obtain a complete vanillin recovery. It was determined a decrease of the vanillin concentration in the extract phase along the fractionation process. A complete vanillin recovery was obtained using a solvent mass ratio of 25 g CO_2/g oleoresin processed.

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1. Introduction

Vanilla is a spice whose flavor is the most popular and consumed worldwide. It is used as a flavoring agent in food industry and in the manufacturing of cosmetics and pharmaceuticals. The composition of vanilla beans depends on various factors such as plant species, the growing region, harvesting, and curing technology. The natural vanilla extract has many flavor compounds, more than 170 volatile aromatic components have been identified in trace levels. The main flavor component in a vanilla extract is vanillin (4-hydroxy-3-methoxybenzaldehyde) that represents about 80–90 wt.% of the flavors components. In vanilla beans, vanillin is found at levels of 20 g vanillin/kg beans in a dry weight base [1,2].

There are mainly four traded vanilla products: the aqueous/ethanol extract, the oleoresin, vanilla absolute and vanilla sugar. The most important product commercialized worldwide is the vanilla extract that is conventionally obtained by percolation of an aqueous ethanol solution at 30–40 vol.%. This process requires between 48 and 72 h to obtain a complete extraction of the mayor flavor components and a subsequent concentration of the

extractable material by solvent evaporation under vacuum. One of the main concerns of the conventional extraction process, besides long extraction time periods, is the temperature. A poor control of this variable could result in degradation of the vanilla oleoresin extract [2,3].

New methods of extraction and fractionation of natural products that contemplate consumer preferences, regulatory controls, and that are cost-effective, are becoming increasingly popular. One of the major technologies that have emerged in recent decades as the alternative to the extraction of natural products with traditional organic solvents is the supercritical fluid fractionation. This technology uses solvents such as carbon dioxide that are clean, safe, cheap, non-flammables, non-toxic, and environmentally friendly. In addition, energy costs associated with this new technique of extraction/fractionation could be lower than the costs of traditional methods of organic solvent extraction that are subjected to worldwide mandatory regulations. The supercritical CO_2 technology is becoming more important over conventional techniques of extraction, fractionation and purification of natural products [4].

The supercritical CO_2 (SC-CO_2) extraction of vanilla oleoresin has been previously studied by different authors [5–7] to produce a new absolute vanilla extract that is concentrate in vanillin. Nguyen et al. [5] studied the extraction of vanilla oleoresin at 110 bar and 36 °C obtaining extraction yields as high as 10.5 wt.% (oleoresin/dry

vanilla beans). According to the authors, this yield was comparable to the yields obtained in the conventional ethanol/water extraction. However, long extraction times (160 h) were required to reach a complete extraction. Vitzthum and Huber [6] worked in the extraction of crushed vanilla pods at 400 bar and temperatures between 298 K and 318 K and obtained a wet precipitate with a 28 wt.% of vanillin content in 6 h of extraction time. Schütz et al. [7] carried out the extraction of vanilla oleoresin with CO_2 at 160 bar and 298 K obtaining an extract product with 98.5 wt.% of the initial vanillin present in the vanilla pods.

A proper design of an extraction/fractionation process of vanilla oleoresin also requires an analysis of the oleoresin solubility and a selectivity study about the preferential extraction of vanillin (main flavor active principle) over other components. Binary data of vanillin solubility in CO_2 has been previously reported in the literature [8,9]. However, the vanilla oleoresin is a very complex liquid substrate. Cured vanilla beans normally contains minerals (6 wt.%), cellulose (30 wt.%), water (35 wt.%), sugars (25 wt.%), and up to 15 wt.% of fats whose main fatty acid composition are palmitic and oleic acids. The natural vanilla extract contains acids, lactones, sulphur compounds, ethers, furans, alcohols, carbonyl alcohols/aldehydes/ketones, esters, acetals, phenols, pyrans, and coumarins [10,11]. Therefore, the solubility of the different oleoresin components in CO_2 can be very difficult to predict a priori from binary data because mutual interaction among the different solutes can extraordinarily alter the phase behavior of binary mixtures as showed by Gañan and Brignole [12,13]. These authors studied the fractionation of essential oils with CO_2 and demonstrated that there was a remarkable variation between the relative volatility of the components in the multicomponent mixture respect to the binary systems [12].

In this work, it was studied the CO_2 fractionation of a viscous vanilla oleoresin liquid substrate obtained by percolation of ethanol through chopped vanilla pods (*Vanilla planifolia*). Firstly, it was determined in a dynamic high pressure cell the initial vanilla oleoresin solubility at moderated pressures (140–180 bar) and temperatures (310.5–348.3 K). A liquid oleoresin substrate was used in the experiments to avoid mass transfer problems during the dynamic CO_2 extraction. The vanillin content in the extract product was analyzed to evaluate how temperature and pressure modify the selectivity. An important variation of the oleoresin concentration (particularly of vanillin) was observed in the CO_2 extract phase with temperature and pressure. The solubility of oleoresin and vanillin in CO_2 were correlated by a dimensionless version of the del Valle–Aguilera equation [14,15]. Secondly, it was also studied the fractionation process at 310.5 K and 140 bar to determine experimentally the solvent mass ratio (CO_2/oleoresin) required to obtain a complete vanillin recovery. Also, it was evaluated the variability of the vanillin concentration in the CO_2 extract phase along the fractionation process.

2. Materials and methods

2.1. Materials

Liquid, food grade (99.8 wt.%) CO_2 from Air Liquid (Bahía Blanca, Argentina) was used as solvent in the experiments. Pelargonic acid methyl ester (99.9 wt.%, Sigma Aldrich, Argentina) and vanillin (99.9 wt.% Sigma Aldrich, Argentina), were used as analytical standards in GC analyzes. Ethanol (99.9 wt.%, GC grade), *n*-hexane (98 wt.%, technical grade), and acetone (99.9 wt.%, GC grade) were used as solvents in the experiments and GC determinations.

The vanilla oleoresin used in experimental studies was obtained according to techniques reported in previous works [2,3,10]. Dry and cured vanilla beans (*V. planifolia*) were chopped in 3 mm

regular pieces and then were extracted by hot ethanol of technical grade for 12 h in a Soxhlet apparatus. Ethanol (95.6 wt.%) was used instead of the conventional water/ethanol solutions in order to favor the evaporation of the solvent minimizing the loss of vanillin from the vanilla oleoresin. The solvent was evaporated in a roto-evaporator operated at 60 °C, under vacuum, and with a gentle nitrogen stream. An extraction yield of 37.2 wt.% (oleoresin/initial vanilla beans) was obtained. A GC analysis of the vanilla oleoresin used in the solubility and supercritical fractionation studies indicated a mean content of 5.9 wt.% (vanillin/vanilla oleoresin).

The vanilla pods were also extracted for 12 h with technical grade hexane in a Soxhlet apparatus and it was obtained a 14.2 wt.% of liposoluble components (free vanillin basis). According to previous studies [2,6,10,16], this is a liposoluble fraction that can be partially extracted by CO_2 and is composed by unsaturated mono-carbonyl, dicarbonyl compounds, and unsaturated pyranones. They were arbitrary named in this work as "vanilla oleoresin lipids".

2.2. Experimental equipment and procedure

Fig. 1 shows the experimental setup used in this work to determine the vanilla oleoresin solubility and to study the CO_2 fractionation of vanilla oleoresin. It consists of a high pressure dynamic extraction cell described previously [12] that was adapted to achieve the experimental goals proposed in this work. The extraction cell (SS-316) has 46.9 mL capacity (id.: 18 mm), and a maximum operating temperature and pressure of 80 °C and 300 bar, respectively. The equipment was assembled with a gas sample cylinder of 500 mL for the storage of liquid CO_2. The system was pressurized by an HPLC pump with a refrigerated head (2 °C). A recirculation loop with a back pressure regulator was used to keep a constant pressure and solvent flow rate in the extractor. A thermostatic air bath equipped with a heating controller was used to control the operating temperature in the high pressure cell. The solvent flow rate (0.18–0.63 g/min) was controlled by a metering valve that was heated by electric cords to avoid the CO_2 clogging during the solvent expansion process at atmospheric pressure. A glass separator, refrigerated at 277 K, was used to separate and collect the CO_2 extract.

A second extraction column (3 mL, capacity) filled with acetone was set in parallel to the main extractor. This column was useful to determine properly the pure vanillin and oleoresin solubility in CO_2 because it enables the extraction, cleaning and collection of the oleoresin that remains deposited in the metering valve and in the discharge line after the depressurization process.

The experimental procedure for solubility measurements (pure vanillin or vanilla oleoresin) was the following. Pure vanillin ($\approx$1 g) or vanilla oleoresin extracted by hot ethanol ($\approx$5 g) was mixed with glass beads (d: 1 mm–$\approx$68 g) and then placed inside the extraction column. Defatted cotton was put at both ends of the extractor to avoid the substrate entraining in the system. After the extractor assembling, the system was purged with CO_2 at ambient conditions and then pressurized (140 and 180 bar) at the operating temperature (310.5, 328.3 and 348.3 K). The entire system was left for 60 min to reach equilibrium at constant operating conditions. Afterwards, the extraction was initiated by turning on the HPLC pump. It was regulated at a solvent flow rate barely higher than the CO_2 flow controlled by the metering valve at the extractor outlet (0.18 g/min). The recirculation loop set at the operating pressure allowed keeping constant the operating conditions during the dynamic extraction. The substrate extracted by CO_2 was precipitated after the solvent expansion at atmospheric pressure, and it was collected in glass traps refrigerated at 277 K. The CO_2 flow rate was recorded by a gas flow meter (brooks 4800) and vented to the ambient. After 60–90 min of continuous operation, the extraction cell was isolated at both ends closing the
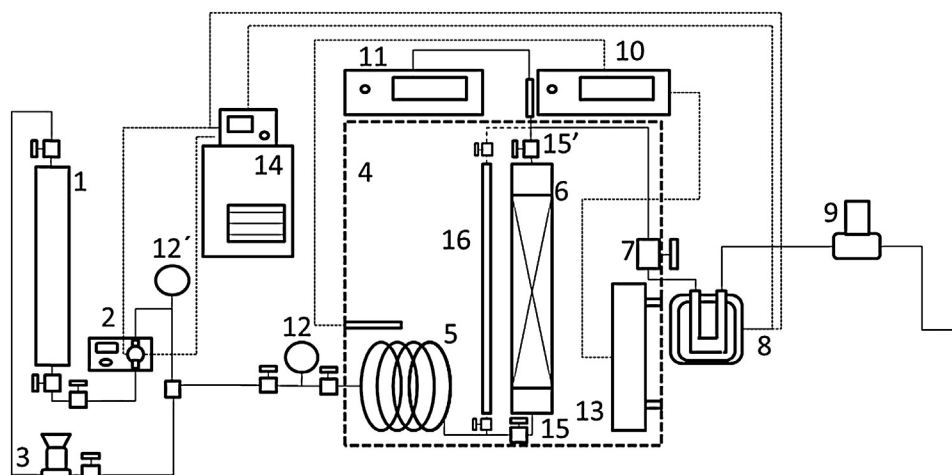
Fig. 1. Experimental setup used in the CO_2 vanilla oleoresin solubility measurements. (1) Gas stainless steel sample cylinder, (2) HPLC pump, (3) BPR valve, (4) air thermostatic bath, (5) heating coil element, (6) extraction vessel, (7) needle valve, (8) refrigerated separator, (9) flow-meter, (10) temperature controller, (11) extractor temperature indicator, (12) pressure sensors, (13) heating element, (14) low refrigerated circulator, (15–15′) on–off valves of extractor vessel.

appropriate on–off valves. In order to determine properly the solubility, a washing column loaded with solvent (2.5 mL acetone) was used to clean the metering valve/line and to collect the residual substrate deposited during the CO_2 expansion. To measure pure vanillin solubility, the solution obtained at the extractor outlet in the glass trap was roto-evaporated to quantify the mass of vanillin extracted. To evaluate the solubility of vanilla oleoresin and its vanillin content, the solution collected in the glass trap was diluted up to 25 mL in a volumetric flask. A sample of this solution (1 mL) was reserved for GC analysis in order to quantify the vanillin content. Then, acetone was roto-evaporated under vacuum at 318 K to determine the oleoresin concentration in the solution and quantify gravimetrically the total amount of vanilla oleoresin extracted.

The experimental measurements of solubility (pure vanillin solubility/initial vanilla oleoresin) at a given temperature and pressure were carried out by triplicate according to three consecutive experiments of a substrate's sample loaded to the extractor. The mean standard deviation of the oleoresin concentration in the extract was ±0.6 g oleoresin extracted/kg CO_2 due to errors associated to gravimetrical measurements and CO_2 quantification. This standard deviation, together with the GC analysis deviations (0.9 wt.%, g vanillin/g oleoresin) for the vanillin quantification in the extracted oleoresin, are pointing out a mean error of 0.3 g vanillin/kg CO_2, which is equivalent to a relative error barely lower than 10% in the CO_2 vanillin solubility.

A similar procedure to the one described for solubility measurements was carried out to study the CO_2 vanilla oleoresin fractionation. However, in order to study how to obtain a complete vanillin recovery along the fractionation process, in these experiments the operating conditions were fixed at 140 bar and 310.5 K, the CO_2 flow rate was 0.18 g/min and 0.63 g/min, and the amounts of vanilla oleoresin processed were 3 g and 5 g. Also, the extraction-washing cycle ($\approx$60 min equilibrium time/CO_2 dynamic extraction/acetone washing) was repeated by "n" stages for a given mass of vanilla oleoresin (3 g and 5 g) loaded to the high pressure extraction cell.

As indicated previously, the initial concentration of vanillin in the vanilla oleoresin processed was determined by GC (5.9 wt.% vanillin/oleoresin). The initial concentration of vanilla oleoresin lipids was estimated from hexane liposoluble compounds (14.2 wt.% vanillin/dry vanilla pods, free vanillin basis) obtained in a Soxhlet extraction. Thus, the oleoresin processed has 41.9 wt.% of oleoresin lipids and 52.2 wt.% of CO_2 non-soluble oleoresin compounds.

2.3. GC-analysis

The vanillin concentration in the CO_2 oleoresin extract was determined by gas chromatography in a GC Varian star 3400 CX assembled with a FID detector, a split/splitless injector and capillary column (J&W Scientific, model DB-5ht, 15 m length, 0.32 mm inner diameter, and 0.10 μm film thickness). The injector and detector temperatures were 593 and 638 K, respectively. The oven temperature program was initially set at 323 K for 1 min and included a ramp of 15 K/min to 453 K, a ramp of 7 K/min to 503 K, and a ramp of 10 K/min to 631 K, where the temperature was maintained for 10 min.

A stock solution of nonanoic acid methyl ester in ethanol (6 mg/mL) was used as internal standard. A stock pure vanillin solution in ethanol was used to prepare a calibration curve of vanillin against the internal standard to determine the vanillin concentration in the oleoresin sample solution (obtained in the fractionation process). In a typical analysis the internal standard solution (100 μL) was mixed with the sample solution (400 μL) and 1 μL of the final solution was injected to the GC. Triplicated analysis of the samples showed an experimental mean standard deviations of 0.9 wt.% of the vanillin concentration present in the oleoresin extract (vanillin/oleoresin %).

3. Modeling

3.1. Oleoresin solubility

The solubility of vanillin and vanilla oleoresin in CO_2 were correlated by the semi-empirical model proposed by del Valle-Aguilera [14] to fitting the solubility of vegetable oils in CO_2. It was used a dimensionless version of this equation that was suggested more recently by Sparks et al. [15] (Eq. (1)) to correlate the solubility of solids in supercritical CO_2. In this case, the model was useful to fit the oleoresin solubility because it was not necessary to estimate critical parameters and acentric factors of the several components present in the vanilla oleoresin. This type of model has also been applied successfully to correlate the CO_2 solubility of different lipids and derivatives as fatty acids, monoglycerides, diglycerides and triglycerides [17].

The solubility measurements of pure vanillin $\left(y_1''\right)$ and vanilla oleoresin $\left(y_{\text{oleoresin}}^*\right)$ were modeled by Eqs. (1) and (4), respectively. The parameters of the model for pure vanillin solubility

were obtained from previous experimental data reported in the literature [8,9]. The model was useful to analyze and compare the experimental data obtained in this work with previous data [8,9] in order to validate our experimental setup.

The oleoresin solubility measurements were correlated from equations Eqs. (2)–(4) in line with the following considerations. The oleoresin obtained in the CO_2 extraction is mainly composed by vanillin and other components. These non-identified compounds according to the literature [6,16] can be described as a liposoluble fraction (named in this work as vanilla oleoresin lipids). Thus, the oleoresin solubility in CO_2 was modeled from the addition of these two terms or contributions that were analyzed individually and represented by the CO_2 solubility of vanillin and oleoresin lipids, respectively. The solubility of vanillin in the CO_2 oleoresin extract $\left(y_1^*\right)$ was estimated from y_1'' by Eq. (2). On the other hand, the solubility of the liposoluble compounds in the CO_2 oleoresin extract $\left(y_2^*\right)$ was estimated from Eq. (3) according to a second set of model parameters. The best-fit model parameters were estimated by minimization of the root mean square % deviation between the modeling results and the experimental data.

$$y_1'' = \left(\frac{\rho_c}{\rho}\right)\left(\frac{\rho}{\rho_c}\right)^k \exp\left(\alpha_1 + \frac{\beta_1}{T_r} + \frac{\gamma_1}{T_r^2}\right) 1000 \tag{1}$$

$$y_1^* = y_1''\left(\delta + \eta T_r\right) \tag{2}$$

$$y_2^* = \left(\frac{\rho_c}{\rho}\right)\left(\frac{\rho}{\rho_c}\right)^k \exp\left(\alpha_2 + \frac{\beta_2}{T_r} + \frac{\gamma_2}{T_r^2}\right) 1000 \tag{3}$$

$$y_{\text{oleoresin}}^* = y_1^* + y_2^* \tag{4}$$

where y_1'' is the pure vanillin solubility, g/kg CO_2; y_1^* is the vanillin solubility in the CO_2 oleoresin extract, g/kg CO_2; y_2^* is the oleoresin liposoluble compounds solubility in the CO_2 oleoresin extract, g/kg CO_2; $y_{\text{oleoresin}}^*$ is the total oleoresin solubility, g/kg; ρ_c is the CO_2 critical density, g/cm^3; ρ is the CO_2 density at operating conditions, g/cm^3; T_r is the reduced temperature, K; K, α, β, γ is the dimensionless parameters.

3.2. Oleoresin fractionation

As mentioned previously, the extraction/fractionation process was performed as "n" successive cycles of extraction–collection. Therefore, the system was modeled as a succession of several liquid–vapor equilibrium contact stages. In each equilibrium stage, the extract phase is sampled out of the system and this promotes the fractionation of the oleoresin that remains in the extractor. The global composition inside the extraction vessel is changing during the fractionation process due to a decrease of vanillin and oleoresin lipids in the raffinate or solid phase.

The model is described as follows. For the equilibrium data, the solute (vanillin: $j = 1$; lipidic fraction: $j = 2$) equilibrium concentration in SC-CO_2 is assumed to follow Nernst distribution law with a saturation in a critical raffinate or solid phase concentration (x_c),

$$y_{eq,j} = y_j^* \quad \text{if} \quad x_j > x_{cj} \quad j = 1, 2 \tag{5a}$$

$$y_{eq,j} = K_{eqj}x_j \quad \text{if} \quad x_j < x_{cj} \quad j = 1, 2 \tag{5b}$$

where $y_{eq,j}$ is the solute mass fraction respect to CO_2 mass (g solute/kg CO_2) in the supercritical phase and x_{cj} is the critical solute mass fraction in the solid phase (g solute/kg inert material).

The total solute recovered at the end of "n" stages (assuming that SC-CO_2 is saturated at the extractor outlet) is given by Eq. (6):

$$m_j = \sum_{i=1}^{n} Y_{eq,j}m_{CO_2 i} \quad j = 1, 2 \tag{6}$$

where m_j is the total mass of solute recovered at the end of given stage; $m_{CO_2 i}$ is the CO_2 mass used in stage i. The mass fraction of solute in the solid phase ($x_{i,j}$) can be calculated by equation Eq. (7):

$$x_{i,j} = \frac{m_j^0 - \displaystyle\sum_{i=1}^{n}Y_{eq,j}m_{CO_2 i} - V_i'Y_{eq,j}}{m_{inert}^0} \quad j = 1, 2 \tag{7}$$

where m_j^0 is the initial amount of solute j, m_{inert}^0 is the mass of inert material (non-soluble oleoresin) and V_i' is the SC-CO_2 that remains in the extraction column that can be calculated by Eqs. (8) and (9):

$$V_i' = \left(V_{total}\varepsilon - \frac{L_i'}{\rho_{raffinate}}\right)\rho_{CO_2} \tag{8}$$

$$L_i' = m_{oleore\,sin}^i\left(1 + x_{CO_2}^{raff}\right) - V_{i-1}'\left(Y_{eq,1} + Y_{eq,2}\right) \tag{9}$$

where L_i' is the mass of raffinate at a given stage i, $\rho_{raffinate}$ is the density of raffinate (assumed as oleoresin density), ρ_{CO_2} is the CO_2 density, $m_{oleore\,sin}^i$ is the mass of oleoresin inside the extractor at stage i that is estimated from the initial amount of oleoresin ($m_{oleore\,sin}^0$) and $m_{j,i}$, $x_{CO_2}^{raff}$ is the CO_2 concentration in the liquid oleoresin. Like the continuity of equilibrium line implicates that,

$$K_{eqj} = \frac{y_j^*}{x_{cj}} \tag{10}$$

y_1^* and y_2^* were estimated from initial solubility measurements, the model can be fit to the experimental results with only two parameters (x_{c1} for vanillin concentration, and x_{c2} for vanilla oleoresin lipid concentration), m_1 or m_2 as dependent variables, and the total amount of SC-CO_2 ($m_{CO_2 tot}$) as independent variable. The $m_{CO_2 tot}$ can be calculated by equation Eq. (11):

$$m_{CO_2 tot,n} = \sum_{i=1}^{n} m_{CO_2 i} \tag{11}$$

The model assumes two extraction periods. In a first extraction period the solubility of vanillin and oleoresin lipids remain constant. Then, in a second period the vanillin and oleoresin concentration in CO_2 of both compounds change according to a partition coefficient ($K_j = y_j/x_j$) when it is reached a critical concentration (x_{cj}) in the raffinate. These coefficients ($x_{c,1}$ and $x_{c,2}$) were left as fitting parameters in the model to adjust experimental data. The modeling assumptions are based on experimental results, operating conditions considered in this work (extraction-washing cycle: ≈ 60 min equilibrium time/CO_2 dynamic extraction/acetone washing) and favorable mass transport properties of supercritical fluids.

The model also considers a uniform oleoresin distribution in the spherical glass beads, a perfect mixing pattern flow of the solvent phase, constant physical properties of the fluid phases, and non-significant gradients of temperature and pressure along the extractor. To evaluate the fractionation process, it was assumed that a ≈ 30 wt.% of CO_2 is dissolved in the vanilla oleoresin (raffinate). This CO_2 concentration in the liquid raffinate phase was estimated from CO_2 + fixed vegetable oils systems at similar operating conditions [4,18]. Indeed, similar CO_2 concentrations in the liquid phase were also determined for the oleoresin-capsicum + CO_2 system [19] which is pointing out that it is a reasonable assumption. According to GC analysis of the vanilla oleoresin, the initial vanillin content in the oleoresin processed was 5.9 wt.% (vanillin/vanilla oleoresin). The initial concentration of vanilla oleoresin lipids was estimated as 41.9 wt.% according to the liposoluble component fraction in the vanilla pods (14.2 wt.%) determined in hexane Soxhlet extractions. Therefore, the mass of
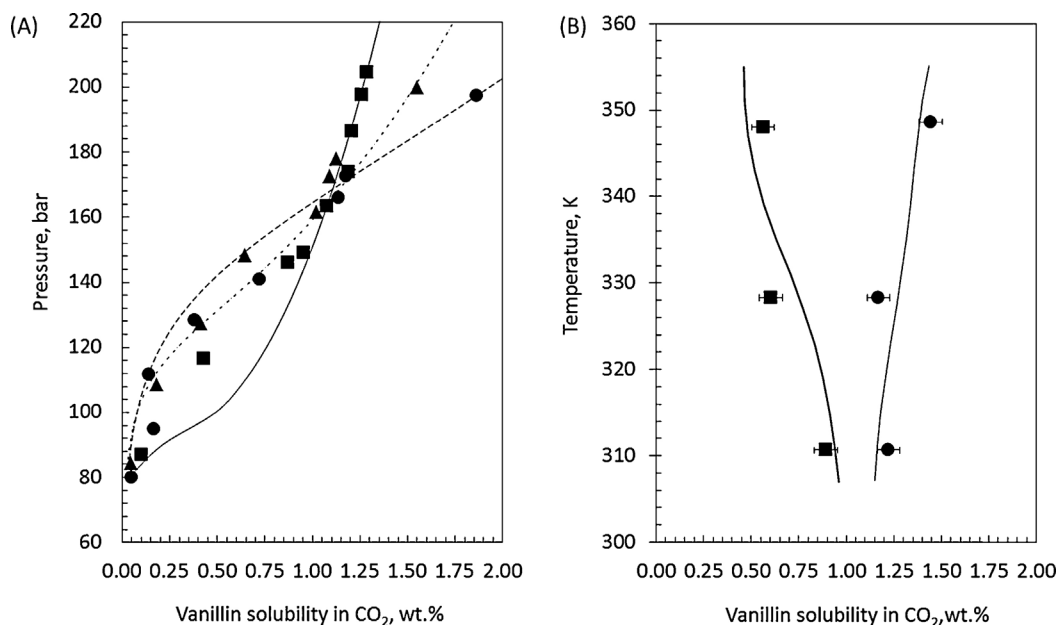
Fig. 2. (A) Pressure vs. vanillin solubility diagram, filled solid symbols are experimental data reported by Skerget et al. [8] at 313 K (■), 333 K (▲), and 353 K (●). Lines are the best fitting lines obtained with modified del Valle–Aguilera model [12,13] according to parameters reported in Table 1 at 313 K (solid line), 333 K (pointed line) and 353 K (dashed line). (B) Temperature-vanillin solubility diagram, symbols are experimental data obtained in this work at 140 bar (■) and 180 bar (●). Lines are the solubility modeling predictions of the del Valle–Aguilera model at 140 bar (dashed line) and 180 bar (solid line) according to parameters reported in Table 1.

non-soluble oleoresin was estimated as 52.2 wt.% of the vanilla oleoresin processed.

The final set of equations was programmed in Excel according to a free-solute mass balance of vanillin and oleoresin lipids between the extract (V'), the raffinate (L') phases, and the extraction phase sample (m_{CO_2}) along the "n" extraction-washing cycles of the fractionation process. The parameters $x_{c,j=1,2}$ were fitted in order to minimize the root mean square % deviation between the modeling results and the experimental data.

4. Results and discussion

4.1. Vanilla oleoresin solubility in supercritical CO_2

Fig. 2 shows the measurements of vanillin solubility in CO_2 that were obtained in this work. It can be observed the results obtained in this study agree well with previous experimental data [8]. Fig. 2b shows vanillin solubility in CO_2 was around 0.9 wt.% at 310.5 K and 140 bar and it decreased to 0.6 wt.% at this isobar and 348.5 K. Also, the vanillin solubility increased due to pressure (180 bar) and this effect was substantially higher at 348.5 K. The concentration of pure vanillin in CO_2 increased at the highest temperature from 0.6 to 1.4 wt.% due to pressure increment. However, only a slightly higher pure vanillin solubility (1.2 wt.%) was measured at 310.5 K and 180 bar (see Fig. 2b). The results are pointing out a greater influence of vanillin vapor pressure over the CO_2 density at the higher pressure studied in this work. The binary vanillin + CO_2 system has been studied in detail by different authors [8,9]. Skerget et al. [8] shows there is an important effect of temperature in the pure vanillin solubility at pressures higher than 160 bar. According to phase transition data reported by Liu et al. [9], in the range of pressure studied in this work (140–180 bar) the binary vanillin + CO_2 system exhibit a solid + gas condition at temperatures lower than 338.3 K and there is a transition to a liquid + gas behavior at 348.3 K. This phenomenon could explain the moderated increment of vanillin solubility in CO_2 observed in this work.

Fig. 2 also shows the best fitting lines of the vanillin data reported by Skerget et al. [8] that were obtained with the
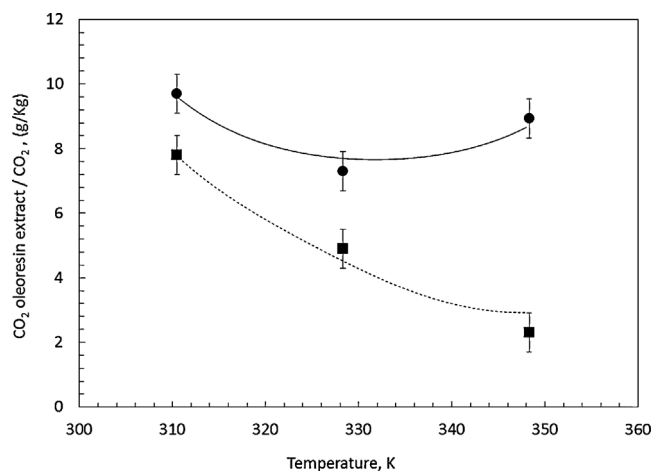

Fig. 3. Oleoresin solubility in CO_2 at different operating conditions. Symbols are experimental data obtained in this work at 140 bar (■) and 180 bar (●). Lines are best fitting lines obtained with the modified version of del Valle–Aguilera model [13] according to parameters reported in Table 1 at 140 bar (dashed line) and 180 bar (solid line).

dimensionless version of del Valle–Aguilera model [15]. It can be observed an acceptable agreement with experimental data for conceptual engineering process design purposes.

Fig. 3 shows the results of vanilla oleoresin solubility in CO_2 obtained in this work. Each point represents the mean value of three consecutive measurements at a given operating condition. It can be observed an important sensibility of the oleoresin concentration to both variables studied. There was a variation of the oleoresin solubility between 2 and 10 g/kg CO_2. A significant reduction of the oleoresin solubility in CO_2 with temperature was observed at 140 bar. However, this effect is less important at 180 bar where there is a slightly reduction of the oleoresin solubility in the extract from 310.5 K to 328.3 K (9.6 to 7.3 g oleoresin/kg CO_2), and then, the solubility increases to ≈8.9 g oleoresin/kg CO_2 at 348.3 K. Fig. 3 also shows the best fitting lines obtained with del Valle–Aguilera

Table 1
del Valle Aguillera model based on Sparks et al. [15] modifications. Model parameters fitted to correlate the solubility of: pure vanillin in CO_2, and vanillin and oleoresin lipids in the CO_2 oleoresin extract.

	Pure vanillin	CO_2 oleoresin extract	
		Vanillin	Oleoresin lipids
k	4.269		4.397
α	52.264		144.865
β	−102.247		−314.870
γ	48.088		162.983
δ		−0.0124	
θ		0.0164	
Mean error by components[a]	32.80%	2.61%[b]	7.83%
Total oleoresin extract error[a]		4.05%[b]	

[a] Root mean square % deviation of the relative error between the model and experimental values.
[b] Error excluding experimental data at 348.3 K and 140 bar.

equation according to parameters reported in Table 1. It can be seen a good agreement between the model and the experimental data. The greater relative error in solubility estimations was observed at 348.3 K and 180 bar. This experimental point was not considered in the model parameters correlation in order to obtain an appropriate assessment of the solubility in the entire range of operation.

Nguyen et al. [5] studied the extraction of oleoresin from vanilla beans using SC-CO_2 at 307 K and pressures between 110 bar and 140 bar. The authors evaluated the effect of extraction time and material pretreatments on the oleoresin yield and its composition. An extraction time of 60 h was used to extract criogrounded beans obtaining a 10.6 wt.% yield (vanilla oleoresin/dried bean) using a solvent mass ratio of 60 g CO_2/g dried bean. The highest vanilla oleoresin concentration in CO_2 reported by Nguyen et al. was 8 g oleoresin/kg CO_2. This value is in good agreement with the oleoresin solubility value determined in this work at 310.5 K and 140 bar.

It is well known, the solubility of a given substrate increases with the CO_2 solvent density. Therefore, an increment in the extraction pressure at a fixed temperature produced an increase of solubility. However, the solubility at a given pressure could also increases with temperature in spite of a minor solvent density because of a higher solute vapor pressure [4,18]. This crossover effect was observed in this work at 180 bar where an increment of temperature from 328.3 K to 348.3 K produced a slight increment of vanilla oleoresin solubility in SC-CO_2.

In the range of CO_2 densities studied in this work (0.4–0.8 g/mL), vanilla oleoresin solubility values obtained in this study were similar to solubility values determined in the literature for different oleoresin substrates. As example, Fernandez-Ronco et al. [19] studied the CO_2 oleoresin-capsicum solubility in a temperature range of 314.7–337.8 K and pressures between 100 bar and 300 bar. The authors found oleoresin solubility values between 1 and 9 g oleoresin/kg CO_2. Danielski et al. [20] studied the marigold oleoresin solubility in supercritical CO_2 at temperatures between 293 K and 313 K and pressures in a range of 120–200 bar. The marigold oleoresin concentration in the extract phase reported by the authors varied with the CO_2 density (0.35–0.8 g/mL) in a range of 0.47–1.7 g oleoresin/kg CO_2.

In the range of operating conditions studied in this work, there are also some similarities between the lipid solubility and the oleoresin solubility in CO_2. Milanesio et al. [21], for example, measured the solubility of *Yarrowia lipolytica's* lipid in supercritical CO_2 at temperatures and pressures between 313 K to 333 K and 140 to 230 bar, respectively. The authors determined a lipid concentration between 2 and 10 g lipid/kg CO_2. The lipid solubility is sensible to the substrate composition; in CO_2-acylglycerides systems the
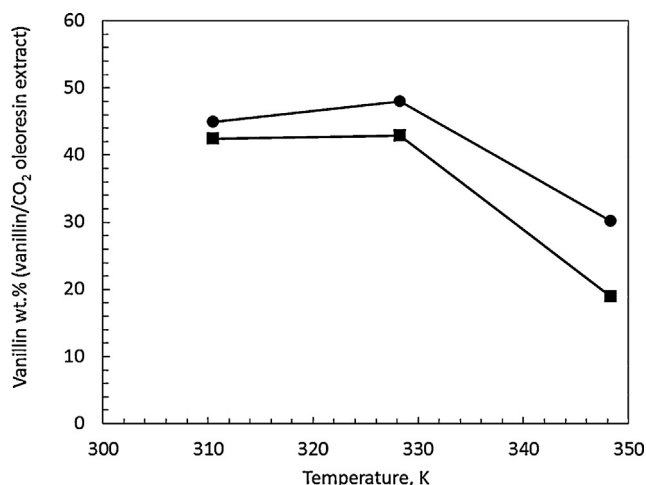

Fig. 4. Vanillin concentration (free-CO_2) in the oleoresin extract obtained at 140 bar (■) and 180 bar (●) for 310.5 K, 328.3 K and 348.3 K.

solubility normally decreases with the molecular weight and polarity [17,22]. Nilsson et al. [23] analyzed the solubility of "olein glycerides" of different polarity in supercritical CO_2 and determined at 150 bar and 323 K a solubility of 2.2 g monoolein/kg CO_2, 1.6 g diolein/kg CO_2, while triolein was not soluble in the CO_2 extract phase. Ashour and Hamman [24] measured the solubility of mono, dilaurin and trilaurin at 313 K and 150 bar obtaining values of 3.3 g monolaurin/kg CO_2, 19.6 g dilaurin/kg CO_2 and 7 g/kg CO_2 trilaurin, respectively.

Even though vanilla oleoresin solubility is a relevant parameter in the extraction/fractionation process, it is also relevant to consider the selectivity toward the extraction of main active components along the process [4,18] to improve the quality of the final product. There is a high concentration of liposoluble compounds in the vanilla oleoresin processed (41.9 wt.%, oleoresin lipids/vanilla oleoresin) that according to the literature [18,23,24] could be soluble in CO_2. Therefore, it is important to analyze the vanillin concentration (main flavor component) in the CO_2 oleoresin extract to evaluate the performance of the extraction at the different operating conditions reported previously.

Fig. 4 shows the vanillin concentration in the CO_2 oleoresin extract (CO_2-free basis fraction) obtained at different operating conditions. It can be observed a significant variation of the vanillin content in the extraction products with temperature. There is a vanillin concentration greater than 40 wt.% (vanillin/CO_2 oleoresin extract) at temperatures between 310.5 K to 328.3 K and 140 bar and it is considerably reduced to less than 20 wt.% at 348.3 K and 140 bar. A pressure increase up to 180 bar produces a greater vanillin concentration in the CO_2 oleoresin extract. However, regarding the vanillin concentration in the extract products the pressure effect is less important than temperature. The most significant difference in the vanillin content due to the pressure increment was determined at 348.3 K where the vanillin content increases from 19 wt.% to 30 wt.%. Moreover, it can be observed there are no significant differences in the vanillin content of the CO_2 oleoresin extract with pressure at lower temperatures. The results indicate at 348.3 K the quality of the CO_2 oleoresin extract is reduced due to a greater extraction of liposoluble components.

Vitzthum et al. [6] extracted crushed vanilla beans with SC-CO_2 at 318.5 K and 400 bar in a two steps process consisting of circulation of dry CO_2 during 4 h followed by a second step where "wet" CO_2 was pumped during 6 h. The authors obtained a net yield of 13.5 wt.% (oleoresin extract/dry vanilla beans) and vanillin concentrations in the oleoresin extract lower than 28 wt.% (vanillin/oleoresin extract). Nguyen et al. [5] carried out
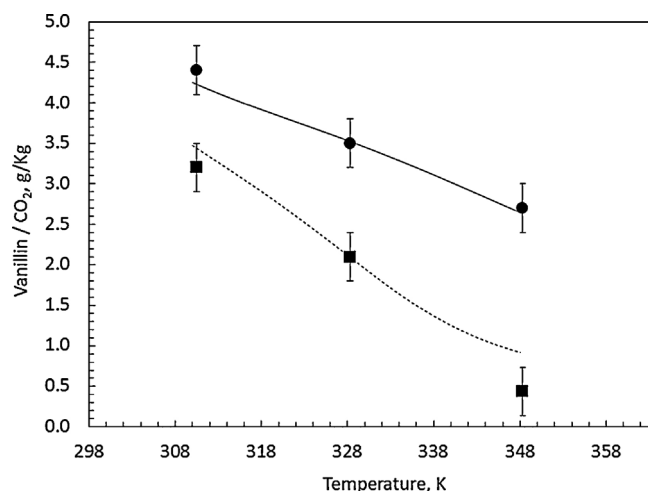
Fig. 5. Vanillin concentration in the CO_2 oleoresin extract phase at different operating temperatures and 140 bar (■), 180 bar (●). Lines are best fitting results of modified del Valle–Aguilera model at 140 bar (dashed line) and 180 bar (solid line) according to parameters reported in Table 1.

Fig. 6. Vanilla oleoresin lipids concentration of in CO_2 extract phase obtained at different operating temperatures and 140 bar (■), 180 bar (●). Lines are best fitting results of modified del Valle–Aguilera model at 140 bar (dashed line) and 180 bar (solid line) according to parameters reported in Table 1.

extractions at 120 bar and 309 K producing CO_2 oleoresin extracts with vanillin contents between 16 wt.% and 36 wt.% (vanillin/oleoresin extract) and an extraction yield ≈10.6 wt.% (oleoresin extract/dry vanilla beans). Schütz et al. [7] used liquefied carbon dioxide to carry out the extraction of vanilla pods working at 160 bar and 300 K. The authors obtained 98–99 g/g % of the total vanillin present in the sample and its concentration in the oleoresin extract was substantially higher (120–140% greater) than the conventional extract obtained with ethanol. The differences in the vanillin concentration of the CO_2 oleoresin extracts reported in the literature could be related with selectivity modifications according to the operating conditions. Vitzthum et al. [6] worked at greater pressures than the other authors obtaining a lower vanillin concentration in the extract. However, this lower concentration can also be explained by variations of the vanilla oleoresin solubility and selectivity toward vanillin along the fractionation process, as explained later in the next section.

Fig. 5 shows the vanillin concentration in the CO_2 extract phase as a function of the extraction temperatures for the two isobars studied in this work (CO_2 + vanilla oleoresin system). It can be clearly seen the vanillin solubility decreases in the extract phase almost linearly with temperature for both operating pressures. This effect is more important at the lowest pressure. The solubility of vanillin in CO_2 at 310.5 K exhibits similar values at both pressures (3.3 g/kg CO_2 at 140 bar against 4.4 g/kg CO_2 at 180 bar). However, at higher temperatures the pressure effect is more relevant, for example the vanillin solubility in the CO_2 extract phase at 348.3 K was 2.7 g/kg CO_2 at 180 bar and it was 0.4 g/kg CO_2 at the lower pressure.

At a given operating condition, the vanillin solubility in CO_2 for the binary system (pure vanillin-CO_2) is higher than the vanillin solubility observed in the vanilla oleoresin-CO_2 system. Also, an opposite behavior of the vanillin concentration in CO_2 can be observed for the vanilla oleoresin-CO_2 system respect to the binary system at 180 bar. Vanillin solubility in CO_2 for the binary system increases with temperature. On the other hand, it was observed at 180 bar a lower vanillin solubility in the CO_2 extract phase due to temperature for the vanilla oleoresin-CO_2 system.

Fig. 6 shows the vanilla oleoresin lipids concentration (liposoluble fraction) in the CO_2 extract phase against the operating temperature for the two isobars studied in this work. This figure was obtained from a mass balance using experimental data reported in Figs. 3 and 5. Similarly to the vanillin solubility behavior, the oleoresin lipids solubility decreases with temperature from
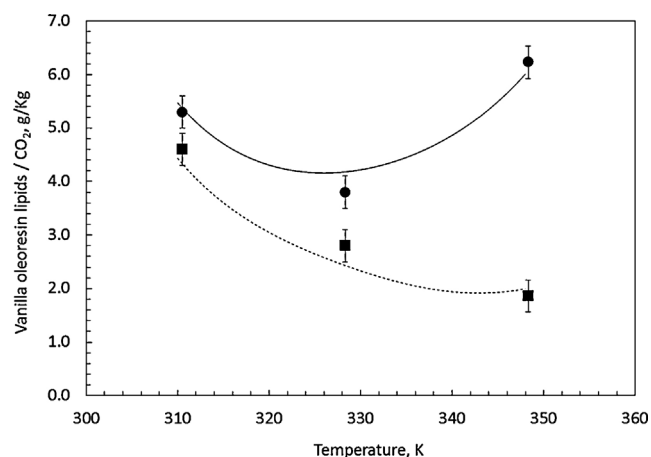
310.5 K to 328.3 K for both operating pressures. However, the vanilla oleoresin lipids concentration in the CO_2 extract phase at 180 bar and 348.3 K shows a significant increment.

Figs. 5 and 6 also shows modeling results obtained with the modified del Valle–Aguilera model [12,13] according to parameters reported in Table 1. It was decided to model the vanillin solubility and liposoluble compounds in the CO_2 extract system as separated terms to improve modeling results. The sum of both concentrations estimated by the model represents the vanilla oleoresin concentration lines reported in Fig. 3. In general, the model agrees well with experimental data with a ≈4% root mean square deviation, which is an acceptable value for engineering process conceptual design. It is worth mention, it was decided to exclude from the analysis the vanillin concentration obtained at 348.3 K and 140 bar because a very low vanillin concentration in the CO_2 extract phase was determined under these operating condition and it introduces a great perturbation in the model.

It can be concluded from the results the vanilla oleoresin extraction/fractionation process should be carried out at low temperatures where there is a good selectivity toward vanillin and it is obtained acceptable extraction yields at both operating pressures. In the range of operating conditions studied in this work at higher temperatures, higher pressures were required to have good extraction yields. Therefore, it was decided to continue the study evaluating the extraction/fractionation process at 310.5 K and 140 bar. The lower pressure was selected regarding economic aspects, good extraction yields and selectivity.

4.2. Supercritical CO_2 vanilla oleoresin fractionation

In order to study the supercritical CO_2 extraction/fractionation process of vanilla oleoresin in greater details it was decided to evaluate its extraction at 310.5 K and 140 bar. It was analyzed the effect of solvent mass ratio (CO_2/initial oleoresin, g/g), initial vanilla oleoresin loaded to the extractor (3 g and 5 g), and mass flow-rate (0.18 g/min and 0.63 g/min) on the extraction yield and composition of CO_2 oleoresin extract.

Fig. 7 shows the results obtained in the fractionation studies in terms of the cumulative extraction curves of CO_2 oleoresin extract and vanillin against the CO_2 mass used in the process. It shows the extraction results obtained by processing ≈3 g and 5 g of vanilla oleoresin. The initial slope of both cumulative extraction curves (CO_2 oleoresin extract and vanillin), up to ≈40 g of CO_2, converged to single curves independently of the mass of oleoresin processed (≈3 g
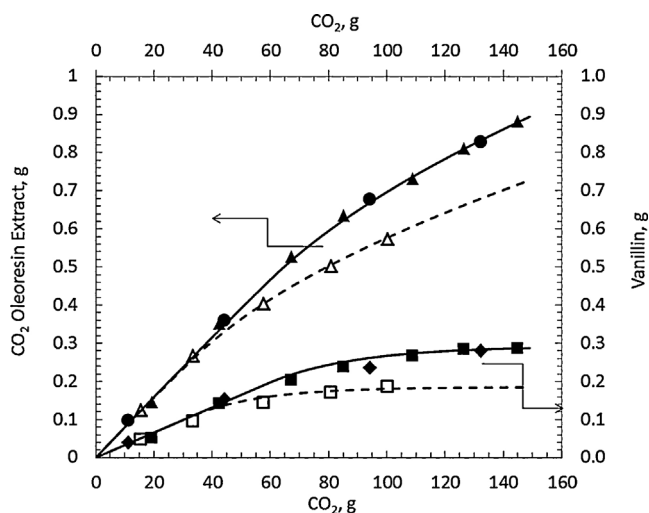
Fig. 7. Cumulative extraction curves of CO_2 oleoresin extract and vanillin in the CO_2 extract as a function of the mass of CO_2. Extraction carried out at 310.5 K and 140 bar for different CO_2 flow rates and initial mass of oleoresin processed. Empty symbols correspond to ≈3 g of oleoresin: (□) vanillin; (△) CO_2 oleoresin extract, 0.18 g/min CO_2 flow. Filled symbols correspond to ≈5 g of oleoresin: (■) vanillin, (▲) CO_2 oleoresin extract, 0.18 g/min (♦) vanillin, (●) CO_2 oleoresin extract, 0.63 g/min CO_2 flow. Lines correspond to the equilibrium fractionation model according to the best fitting model parameters reported in Table 2.

and ≈5 g). The slope of this curves correspond to vanillin and CO_2 oleoresin extract solubility. In agreement with the previous measurements, it was determined a maximum concentration of ≈8 g oleoresin extract/kg CO_2, and a maximum vanillin concentration in the CO_2 extract of ≈3.3 g vanillin/kg CO_2 (≈41.2 g/g % CO_2 free basis). The cumulative extraction curves for ≈3 g and ≈5 g of initial vanilla oleoresin processed diverge after 40 g of CO_2 used in the extraction. Indeed, it was found a clear vanillin reduction in the extract phase after ≈40 g of CO_2 for both initial amounts of vanilla oleoresin processed. As expected, this effect was more important for ≈3 g oleoresin rather than ≈5 g because a lower total mass of vanillin was present in the extraction vessel (0.186 g vanillin in 3 g oleoresin against 0.287 g vanillin in 5 g oleoresin). However, these differences in the cumulative extraction curves are not necessarily indicating that the system is out of equilibrium. Fig. 8 shows
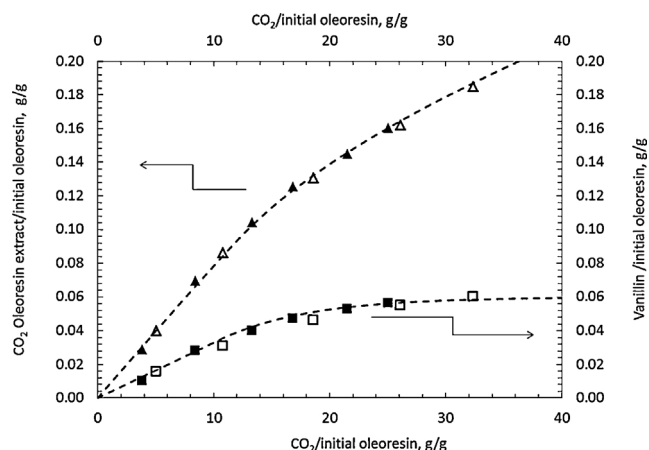

Fig. 8. Cumulative extraction curves of CO_2 oleoresin extract/initial oleoresin and vanillin/initial oleoresin as a function of the solvent mass ratio (CO_2/initial oleoresin processed). Extraction carried out at 310.5 K and 140 bar, 0.18 g/min CO_2 flow. Empty symbols correspond to 3.095 g of vanilla oleoresin: (□) vanillin; (△) CO_2 oleoresin extract. Filled symbols correspond to 5.051 g of oleoresin: (■) Vanillin, (▲) CO_2 oleoresin extract. Lines correspond to the equilibrium fractionation model according to the best fitting model parameters reported in Table 2.

Table 2
Supercritical CO_2 fractionation of vanillin from vanilla oleoresin. Model parameters fitted from experimental data reported in Figs. 8 and 9.

	Oleoresin lipids	Vanillin
$y_j{}^a$ (g solute/g CO_2 extract)	4.7×10^{-3}	3.3×10^{-3}
x_c (g solute/g non-soluble oleoresin)	0.765	0.025
$K_j = y_j/x_j$	0.0061	0.132

[a] Initial concentration of oleoresin lipids and vanillin in the vanilla oleoresin CO_2 extract obtained from solubility measurements (reported in Figs. 5 and 6).

the cumulative extraction yields (g oleoresin extract/g initial oleoresin; and g vanillin/g initial oleoresin) as a function of the solvent mass ratio (g CO_2/g initial oleoresin). It can be seen both extraction curves (CO_2 oleoresin extract and vanillin), even for different initial amounts of vanilla oleoresin processed, converge on single curves.

The effect of CO_2 flow rate in the fractionation process was analyzed for ≈5 g of vanilla oleoresin loaded to the extraction vessel. The mass of CO_2 oleoresin extract converged to a single curve independently of the CO_2 flow (▲, 0.18 g/min and ●, 0.63 g/min). A similar result was observed for vanillin (■, 0.18 g/min and ♦, 0.63 g/min). Therefore, it can be concluded the CO_2 flow used in the dynamic extraction has non-significant effects on the extraction results in the range of operating conditions studied in this work (0.18–0.63 g/min, 140 bar–310.5 K). A greater solvent flow rate implicates a lower residence time (from 81 min to 23 min). However, the increasing flow also increases the external mass transfer coefficient which enables the solvent saturation at the extractor outlet. The results are showing that CO_2 is effectively saturated at the extractor outlet at the operating conditions studied in this work, which agrees with the equilibrium fractionation modeling approach proposed to analyze the process.

An analysis of the process with the fractionation model indicates the system remained in equilibrium and there is a reduction of oleoresin and mainly vanillin in the CO_2 extract phase according to a constant partition coefficient (Table 2). Actually, this kind of phenomena is normal in the fractionation of ternary or multicomponent liquid substrates [4,22]. As an example, Soto et al. [25] shows for the ternary mixture of fatty esters, acylglycerides and CO_2 that during the fractionation process, even in equilibrium conditions, the system exhibits a clear reduction of the ester concentration in the extract phase. This fact is related with the depletion of the main component in the raffinate phase with the advance of the extraction process. This phenomenon has also been observed in the supercritical fractionation of essential oils [26] or the deterpenation of citrus peel oils with supercritical solvents [27,28].

Fig. 8 shows it was obtained an extraction yield of 0.125 g CO_2 oleoresin extract/g initial oleoresin at a solvent mass ratio of 16 g CO_2/g initial oleoresin processed. A vanillin extraction yield of 0.047 g vanillin/g oleoresin was obtained at the same solvent mass ratio; which represents the 84 wt.% of the total vanillin content in the oleoresin processed (5.9 g/g %). The mean value of the oleoresin solubility, as well as the vanillin solubility in the CO_2 extract phase can be estimated from the initial slope of the cumulative extraction yield curves for oleoresin and vanillin, respectively. It can be observed a significant reduction of the oleoresin solubility for a CO_2 mass ratio greater than 17 g CO_2/g oleoresin (from initial slope of 8 g oleoresin/kg CO_2, the solubility is reduced to 4 g oleoresin/kg CO_2). This effect is related to depletion of the vanillin in the raffinate that modifies the equilibrium in the system.

Fig. 9 shows an analysis of the process for the variation of vanillin (A) and oleoresin lipids (B) in the extract and raffinate phases against the mass of CO_2 used in the fractionation process or its equivalent solvent mass ratio. The curve for the raffinate phase was estimated from a mass balance between the extract phase and
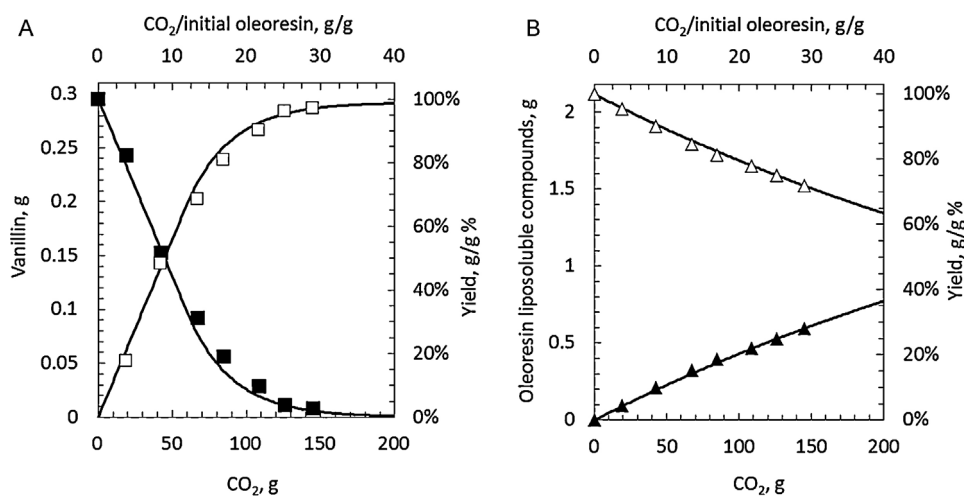
Fig. 9. Extraction curves of vanillin and oleoresin liposoluble compounds against the mass of CO_2 used in the process. Extraction carried out at 310.5 K and 140 bar, 5 g oleoresin, 0.18 g/min CO_2 flow. Vanillin obtained in the (□) extract and (■) raffinate; B. vanilla oleoresin lipids in the (▲) extract and (△) raffinate. Yields correspond to CO_2 extract/total amount of substrate present in the oleoresin. Lines correspond to the equilibrium fractionation model according to the best fitting model parameters reported in Table 2.

the initial content of vanillin (5.9 g/g %, g vanillin/g oleoresin) and oleoresin lipids (41.9 g/g %, g liposoluble compounds/g oleoresin) present in the sample. An analysis of the results (Fig. 9) is indicating it is convenient to finish the extraction at a solvent mass ratio of 17–20 g CO_2/g initial oleoresin because the extract product that is obtained after this solvent mass ratio has a low concentration of the main flavor component. However, the oleoresin concentration in CO_2 at the extractor outlet is still important indicating that liposoluble compounds present in the raffinate phase can still be extracted in the process (a Soxhlet of vanilla pods with *n*-hexane showed 14.2 wt.% of liposoluble compounds in a dry basis, which represent 41.9 wt.% of the vanilla oleoresin obtained by hot ethanol).

The results obtained in this work could explain the different results reported in the literature [4,6] for similar operating conditions respect to the vanillin content in CO_2 oleoresin extracts. It is possible to observe from Fig. 9A, a vanillin recovery of 91% was obtained in the extract phase for 105 g of CO_2 (equivalent to a solvent mass ratio 21 g CO_2/g oleoresin). However, the oleoresin lipids were only partially extracted for the same degree of extraction (only 21% of the liposoluble compounds were obtained for 105 g CO_2). The analysis of the process is pointing out that at a solvent mass ratio of 25 g CO_2/g oleoresin (125 g CO_2) a 95% of the total vanillin can be obtained in the CO_2 oleoresin extract. However, the instantaneous vanillin concentration in CO_2 at the extractor outlet for this solvent mass ratio is reduced in an order of magnitude (0.37 g vanillin/kg CO_2) due to the vanillin depletion in the raffinate. If the extraction proceed, the global vanillin concentration is significantly reduced in the CO_2 oleoresin extract (from ≈40 wt.% to ≈35 wt.% g vanillin/g oleoresin) because 97 wt.% of the initial vanillin has been extracted at 30 g CO_2/g oleoresin.

5. Conclusions

The initial solubility of vanilla oleoresin in supercritical CO_2 was determined at moderated pressures (140 bar and 180 bar) and different temperatures from 310.5 K to 348.3 K. It was observed an important variation of oleoresin and vanillin concentrations in the CO_2 extract according to the operating conditions (2–10 g oleoresin/kg CO_2). Higher values of vanilla oleoresin solubility in CO_2 were obtained at the highest operating conditions. However, the selectivity toward vanillin in the CO_2 extract product decreases with temperature at both pressures studied. The results are pointing out that it is important to operate the extractor at the lowest temperature (310.5 K) to concentrate the CO_2 oleoresin extract in vanillin.

A fractionation study of vanilla oleoresin was carried out at 310.5 K and 140 bar. The CO_2 oleoresin concentration, as well as its vanillin content, in the CO_2 extract phase was constant at the beginning of the extraction process (8 g oleoresin/kg CO_2, and 3.3 g vanillin/kg CO_2) and independent of initial mass of oleoresin processed. However, the concentration of vanillin and oleoresin liposoluble components decreased with the advance of the extraction due to the depletion of vanillin in the system. The results obtained in this study shows the vanilla oleoresin concentration in the extract phase remained constant up to a solvent mass ratio of 16 g CO_2/g oleoresin. At this extraction point, it is possible to recover up to ≈84 wt.% of the initial vanillin present in the system and the CO_2 oleoresin extract exhibit a ≈42 wt.% of vanillin content. Nearly a complete vanillin recovery can be obtained for a solvent mass ratio of ≈25 g CO_2/g oleoresin at the cost of a lower quality in the final CO_2 oleoresin extract (≈35 wt.% vanillin content). A further CO_2 extraction of the vanilla oleoresin from 25 g CO_2/g oleoresin produced an almost free-vanillin oleoresin product indicating it is convenient to stop the extraction.

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