



Solubility measurements and analysis of binary, ternary and quaternary systems of palm olein, squalene and oleic acid in supercritical carbon dioxide

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ABSTRACT

Through the application of a dynamic method, the solubility of three lipid components with different volatilities and polarities was measured in supercritical carbon dioxide (SC-CO₂). Binary systems of CO₂/squalene (SQ), CO₂/oleic acid (OA) and CO₂/pseudo-component palm olein (PO) were measured at temperatures of 313, 333 and 353 K, and at a pressure range of 10–30 MPa. A ternary system of CO₂/SQ/PO and a quaternary system of CO₂/SQ/PO/OA were also investigated at 313 K and pressures of 10–25 MPa. Comparison of the ternary system with the binary system showed a decrease in the solubility of SQ, with a corresponding rise in the solubility of PO. In the quaternary system, the presence of OA decreased the selectivity of SQ. The data were correlated using Chrastil's equation in order to examine the general trend of solubility behaviour.

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1. Introduction

In the chemical and pharmaceutical industries, vegetable oils and their distillates from oil refining are principal sources of valuable components such as squalene, vitamins, sterols and fatty acids. The use of supercritical carbon dioxide (SC-CO₂) to extract these components has been the subject of abundant research in the field [1,2]. Carbon dioxide has been thoroughly investigated as the main supercritical solvent in the extraction of bioactive materials, largely due to it being non-toxic, non-flammable and having mild critical conditions of 304.19 K and 7.3 MPa for temperature and pressure, respectively [3,4]. Reliable information on equilibrium solubility behaviour is essential to obtain basic knowledge regarding the feasibility of supercritical fluid (SCF) separation and fractionation processes. However, the majority of the solubility data are presented as binary systems and there have been relatively few investigations into multi-components systems [5]. Knowledge of these kinds of complex systems is essential for determining the selectivity of a specific solute presented in a mixture, as well as understanding the intermolecular interactions in the solvation process. This investigation is based upon the use of a dynamic method for the measurement of the solubility of three lipid components: squalene (SQ), oleic acid (OA) and (pseudo-

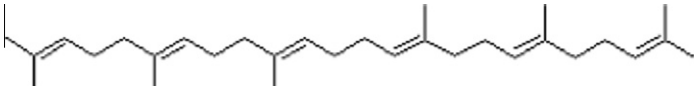
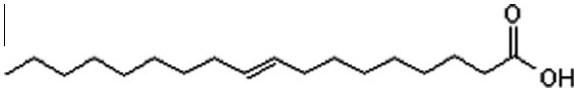
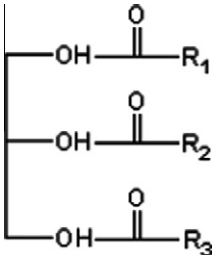
component) palm olein (PO) in SC-CO₂ in binary, ternary and quaternary systems.

Recognition of the health benefits of bioactive lipid components has led to growing interest in understanding the separation and isolation of these components. SQ is an important non-polar hydrocarbon in chemical and pharmaceutical industries. It occurs naturally in fish liver oils and vegetable oils, acting as a precursor for cholesterol biosynthesis [6], as well as enhancing oxygenation of the blood, acting as an anti-cancer agent [7], and being used as a moisturizing or emollient agent in cosmetic preparation [8,9]. As squalene has a very low freezing point (−72 °C), a high boiling point (430 °C at 760 mm Hg), and good thermal and frictional resistance, it has also been used to produce high-grade oil and lubricant for certain scientific instruments [10]. Free fatty acids (FFA) in chemistry are carboxylic acids with a long unbranched aliphatic chain that can be either saturated or unsaturated. Most naturally occurring fatty acids have a chain of 4–28 carbons and are used in many pharmaceutical and nutritional applications, such as soap production and cosmetics [11,12]. The pseudo-component (PO) is widely used as a food oil due to the fact that monoacylglycerol production from palm olein increases advantages and applications in pharmaceutical, cosmetic and food industries [13,14]. The properties and application of these components are summarized in Table 1. This work is concentrated on the fractionation of minor components (less than 3 wt.%) present in deodoriser distillate of palm oil refinery. This work aims to

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Table 1
Summary of properties and applications of squalene (SQ), oleic acid (OA) and palm olein (PO).

Component	Properties		Chemical structure	Typical applications
	Formula	M_w (g/mol)		
Squalene	$C_{30}H_{50}$	410.73		Cholesterol biosynthesis Cosmetics as natural moisturizer Drug Carrier
Oleic acid	$C_{18}H_{34}O_2$	282.46		Soap productions Cosmetics
Palm olein		830 ^a		Food oil Monoacylglycerol production Cosmetic and food industries

^a Pseudo-component palm olein molecular weight is estimated using its saponification value.

examine the solubility of SQ in SC-CO₂ and investigate its solubility in the presence of other lipid components. All multi-component systems are investigated at SQ concentration of 10 wt.% based upon the belief that this is the maximum concentration of SQ that can exist in palm oil deodorisation distillate.

2. Materials and methods

2.1. Materials

Squalene 98%, hexamethyldisilazane (HMDS) 98%, 5- α -cholestane 97% and heptadecanic acid were purchased from Sigma Aldrich. Toluene 99.9% was obtained from Fluka. Ethanol 99.8% and chloroform 99% were purchased from Fisher Scientific. Oleic acid and palm olein were supplied by IOI group (The Netherlands). Carbon dioxide (purity of 99.99%) and helium (purity 99.9%) were obtained from BOC (UK).

Palm olein consists of ~90 wt% triglycerides and ~10 wt% diglycerides. In this study, it was considered as a pseudo component and according to the official method of analysis the average molecular weight was estimated to be 830 g/mol using its average saponification value of 203 mg KOH/gOil [15].

2.2. Experimental set-up

Solubility measurements were conducted by means of a dynamic flow-type apparatus as illustrated schematically in Fig. 1. It is capable of operating at a maximum pressure and temperature of 300 bar and 343 K, respectively.

Liquid carbon dioxide CO₂ (solvent) from a cylinder was passed through 5 μ m filter and then through a cold bath (C) which was fitted before the pump and was maintained at approximately 0–5 °C, ensuring that CO₂ was in liquid phase. Liquid CO₂ was then pumped via air driven pump (AP) (Teledyne Fluid Systems, model 4F64) to the desired process pressure. Three sets of back pressure regulator (BPR)/pressure reducing regulator (PRR) and indicators were used to control and monitor pressure changes. In serial they are; TESCOM BPR (model: 26-1720-22-099) to control and monitor the pressure delivered from pump to system, TESCOM PRR (model: 26-1061-24-039) and GO PRR (model: PR57-1A11020141) with Druck indicator (model: 1407/94-2) to control and monitor vessel pressure. The compressed CO₂ was then preheated with hot water through a shell and tube heat exchanger (H) via a water bath

(Grant Technical Specification, Ltd 6G), thus attaining the desired process temperature. In this investigation all process pressures and temperatures used were above the critical pressure and temperature of CO₂. SC-CO₂ then entered the 500 ml pressure vessel (PV), where phase equilibrium was achieved. Liquid sample (either pure component or mixture) was charged in a specific designed glass sample holder placed in the PV, see Fig. 2. The temperature in the PV was measured using a J type thermocouple. CO₂ was contacted with the sample by bubbling through the liquid phase, and was therefore saturated by the sample. After being saturated with solute, the CO₂ stream was then depressurised across a metering valve (V2) into a collection vessel maintained at a pressure of between 8 and 10 bars. The solute was separated from CO₂ and collected in a beaker placed in this vessel. As the solute free CO₂ left the vessel it was measured by passing through a flow-meter (U.G.I METERS LTD, model, 804741), after which it was vented off from the laboratory. Subsequently, the extract was weighed and analysed. In order to ensure CO₂ saturation, loading measurements were carried out at various solvent flow rates ranged between 0.3 and 1.3 kg h⁻¹. The loading measurements required sufficient amount of sample to be conducted, therefore vegetable oil was used to save the targeted components samples. The system is deemed to have reached equilibrium when no further change was recorded in the solute concentration with the CO₂ flow rate, see Fig. 3. Equilibrium was achieved at solvent flow rate of 0.6–0.9 kg h⁻¹ at which the solvent was saturated with the solute and considered as solubility measurements. As the solvent flow rate was increased (above 0.9 kg h⁻¹), the solubility was decreased due to the insufficient contact time to reach saturation.

Accuracy of pressure measurement is ± 0.05 MPa and for temperature is ± 0.5 K.

2.3. Analytical techniques

Squalene concentrations were measured using Agilent Technologies 6850 series Gas Chromatography (GC) with FID detector and capillary column DB-5 (length 30 m \times diameter 0.25 mm \times film thickness 0.1 μ m). The GC was used in split mode with helium as the carrier gas. The oven temperature was set to 160 °C, followed by a temperature ramp from 160 to 350 °C at a rate of 5 °C/min, totalling a run time of 57 min. The detector temperature was kept at a constant 350 °C throughout. 5- α -cholestane was used as an internal standard.

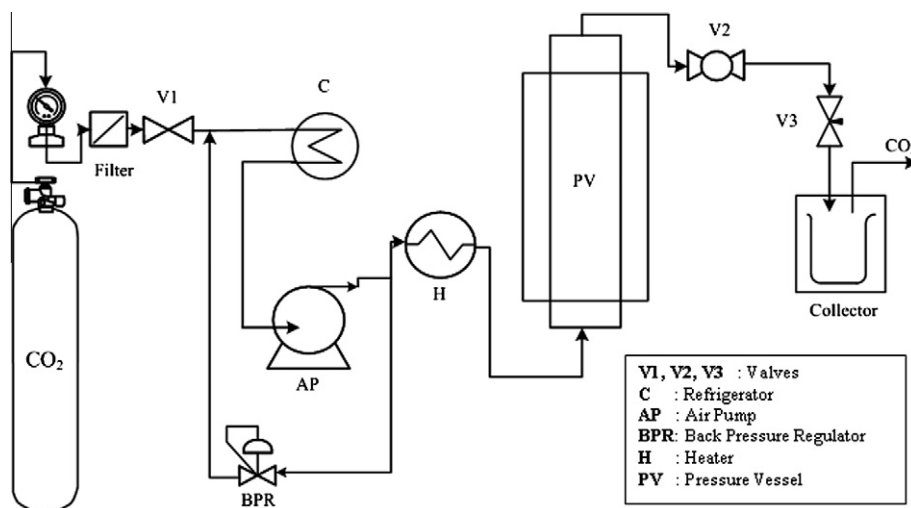


Fig. 1. Schematic of experimental apparatus.

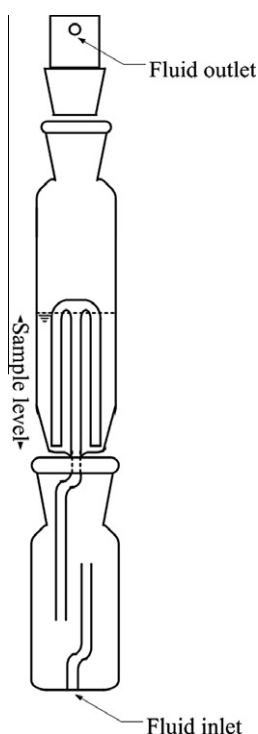


Fig. 2. Liquid sample holder placed in the pressure vessel.

The FFA concentrations were measured using the same GC and column described above for squalene analysis, but using different conditions. The oven temperature programme was held at 100 °C for 3 min, then increased by 10 °C/min to 150 °C, 5 °C/min to 250 °C, 10 °C/min to 350 °C, with a 15 min holding time. The total run time was 53 min. Helium was used as the carrier gas and heptadecanic acid was used as internal standard [16].

3. Results and discussions

3.1. Solubility of binary systems of pure solutes

In order to understand the solubility behaviour in multi-component systems, it is essential to investigate the solubility of the binary systems of the component. The experimental solubility of the

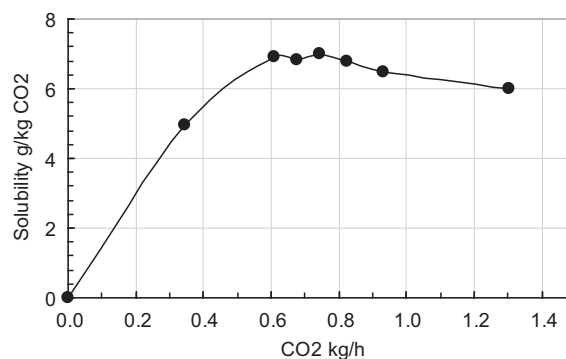


Fig. 3. Loading of vegetable oil at different solvent flow rate at pressure of 15 MPa and temperature of 313 K.

binary systems of OA/SC-CO₂, SQ/SC-CO₂, and PO/SC-CO₂ were measured in the temperature range of 313–353 K and pressure range of 10–30 MPa. To our knowledge, the solubility of the binary system of pseudo-component PO in SC-CO₂ has not been reported in the literature. The solubility results in mole fraction for each component in the binary systems are presented in Tables 2–4 respectively, with each point being the average of a minimum of three measurements. Solubility isotherms for the three lipid components follow similar trends of increasing with rising pressure at constant temperature. In order to assess the reliability of the equipment and method used, OA solubility data were compared with the values found in the literature [17–19] and presented in Fig. 4. The data for oleic acid solubility is in good concurrence with other investigators at pressure range between 10 and 20 MPa. However, at higher pressure a discrepancy of approximately 35% was observed with the data obtained by Chrastil and Skerget. The solubility values reported by Maheshwari deviated significantly from the values found by Chrastil and Skerget and the data gathered by this investigation. This may be due to the impurities present in the reagent, which led to either positive or negative effects. Dobbs has found that when small amounts of entrainer are presenting, solubility in supercritical fluids can be affected by several orders of magnitude [20]. The purity of the reagent used in this work was more than 98%. Another possible cause of these discrepancies is the sampling technique used during solubility measurements. During sampling, entrainment of the solute in the vapour phase can be the cause of a high degree of solubility. The data were correlated using Chrastil's density related empirical equation, in

Table 2
Solubility of OA in mole fraction as function of pressure and CO₂ density.

T (K)	P (MPa)	ρ (kg m ⁻³)	$y \times 10^4$
313.15	10.0	628.61	2.565 ± 0.40
	15.0	780.23	7.499 ± 0.27
	20.0	839.81	8.989 ± 0.57
	25.0	879.49	12.21 ± 0.69
	30.0	909.89	14.07 ± 0.40
333.15	15.0	604.09	2.598 ± 0.10
	20.0	723.68	6.249 ± 0.45
	25.0	786.55	19.68 ± 1.65
	30.0	829.71	22.39 ± 1.20
353.15	10.0	221.60	0.038 ± 0.02
	15.0	427.15	0.192 ± 0.05
	20.0	593.89	3.102 ± 0.10
	25.0	686.22	10.88 ± 1.78
	30.0	745.60	22.00 ± 1.23

Table 3
Solubility of SQ in mole fraction as function of pressure and CO₂ density.

T (K)	P (MPa)	ρ (kg m ⁻³)	$y \times 10^4$
313.15	10	628.61	3.632 ± 0.23
	15	780.23	8.369 ± 0.99
	20	839.81	11.30 ± 0.33
	25	879.49	14.53 ± 0.95
333.15	10	289.95	0.020 ± 0.01
	17.5	676.38	7.354 ± 0.35
	25.0	786.55	8.345 ± 0.68
	27.0	805.42	11.17 ± 2.14
353.15	10.0	221.60	0.017 ± 0.01
	17.5	505.88	2.689 ± 0.13
	25.0	686.22	8.812 ± 1.05
	27.0	712.61	11.47 ± 0.01

Table 4
Solubility of PO in mole fraction as function of pressure and CO₂ density.

T (K)	P (MPa)	ρ (kg m ⁻³)	$y \times 10^4$
313.15	10.0	628.61	0.243 ± 0.06
	15.0	780.23	0.394 ± 0.04
	20.0	839.81	0.578 ± 0.06
	25.0	879.49	1.201 ± 0.20
	30.0	909.89	1.891 ± 0.07
333.15	10.0	289.95	0.020 ± 0.01
	15.0	604.09	0.105 ± 0.01
	20.0	723.68	0.573 ± 0.05
	25.0	786.55	0.677 ± 0.11
	30.0	829.71	1.252 ± 0.16
353.15	15.0	427.15	0.027 ± 0.01
	20.0	593.89	0.236 ± 0.02
	25.0	686.22	0.996 ± 0.05
	30.0	745.60	1.039 ± 0.21

order to examine the general trend of solubility behaviour, as follows:

$$\ln S = k \ln \rho_{\text{CO}_2} + \frac{a}{T} + b \quad (1)$$

In this equation, S denotes the solubility of solute in SC-CO₂ (g kg⁻¹), ρ is the density of CO₂ (kg/m³), T is the temperature (K), a and b are constants, and k is the association number. Densities of CO₂ were obtained from NIST on-line [21]. The fit of Eq. (1) to the experimental data is shown in Fig. 5. It demonstrates that SQ has the highest solubility in CO₂ among the lipid components in this study, while pseudo-component the lowest is shown by PO.

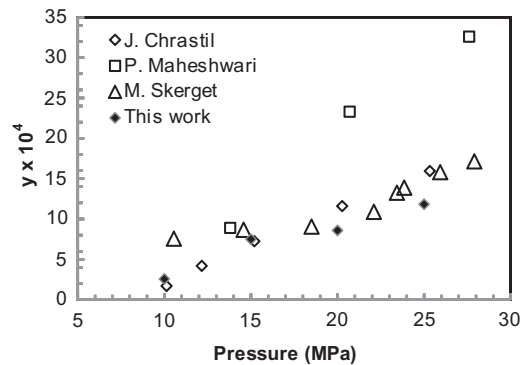


Fig. 4. Solubility isotherms of OA at 313 K.

This result matched expectations, since CO₂ in its supercritical conditions is a non-polar solvent which preferably dissolves non-polar components. SQ is a relatively non-polar hydrocarbon, while a PO structure has hydroxyl groups that enhance its polarity making it relatively more soluble in polar solvents. Meanwhile, OA has a hydroxyl group attached to a non-polar chain, with the result that it is less polar than SQ [22]. Model parameters for each lipid component are given in Table 5 with the average absolute relative deviation (AARD), defined as follows:

$$\text{AARD (\%)} = \frac{100}{n} \sum_1^n \frac{|S_{\text{cal}} - S_{\text{exp}}|}{S_{\text{exp}}} \quad (2)$$

Obtained constants k , b and a were used to calculate the solubility and the calculated solubilities were in good agreement with the measured values with maximum deviation in terms of AARD is 18%, which is lower than the acceptable deviation values of 20% used for empirical equations [23].

3.2. Solubility of solute mixture system

SQ and other lipid components are present in very small concentrations in vegetable oil refinery by-products. A mixture of 10 wt.% SQ in PO was prepared to determine solubility in SC-CO₂ (ternary system) as function of pressure at a constant temperature of 313 K. The solubility data of the ternary mixture (SQ + PO + CO₂) compared with their binary solubility data are shown in Table 6. Selectivity α is calculated as the ratio of respective mole fractions of SQ and PO. It is clear that when PO was added, SQ solubility showed a dramatic reduction, while PO solubility increased at pressures above 15 MPa. The selectivity isotherms for SQ and PO are shown in Figs. 6 and 7. Solubility

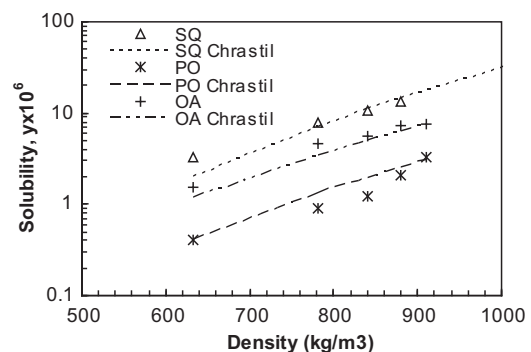


Fig. 5. Solubility of lipid components as function of density at 313 K.

Table 5
Model parameters of Chrastil's equation.

Lipid component	<i>k</i>	<i>a</i>	<i>b</i>	AARD (%)
SQ	6.005	−2543.53	−29.913	12.07
OA	5.056	−3566.41	−21.043	14.08
PO	5.079	−731.212	−31.362	18.26

diminution of a solute has been observed in various studies when another solute is added, as the vapour pressure of the solute is proportional to its concentration in the mixture, as stated in Raoult's law [5].

The behaviour of multi-component systems in SC-CO₂ is poorly understood. These multi-component systems are characterised by further non-ideality and more solute–solute interactions than solute–solvent interactions in binary systems. However, it is possible to highlight that under high pressure the vapour pressure of the highly concentrated PO increases leads to higher solubility, which is aided by the higher interactions with SQ and CO₂ in the ternary mixture leading to further enhanced solubility. This is unlike the afore-mentioned behaviour of squalene, which exists at a much lower concentration. Although SQ solubility decreased dramatically after the introduction of PO into the system, it still dominated the extract composition at the lower pressure range (less than 15 MPa) as the selectivity shows its highest value at low pressures. This indicates that a one step separation of SQ with a concentration of 90 wt.% at a pressure of 10 MPa at temperature of 313.15 K is favoured.

It is clear that when a higher pressure is used, CO₂ will dissolve more PO which effectively dilutes the concentration of SQ in the extract. Catchpole reports similar results, finding that the selectivity toward squalene is best at lower pressure. Solubility data were predicted using Peng Robinson equation for mixture system [7]. Since FFA exists at a relatively high concentration in the deodorisation distillate of palm oil, the solubility behaviour in this work was studied in the presence of FFA for the quaternary systems (SQ + PO + OA + CO₂). The concentration of SQ was kept the same as in the ternary system at 10 wt.%, with addition of 25 wt.% OA and 65 wt.% PO under the same conditions. The solubility data of the quaternary system SQ + PO + OA + CO₂ are provided in Table 7. The extracts from the quaternary mixture of 10% SQ, 25% OA and 65% PO showed that OA has a higher solubility. As OA is more soluble than SQ and PO, the overall amount of solute per kg CO₂ increased. SQ solubility shows relatively little change in the presence of OA in the quaternary system when compared to that in the ternary system, while the solubility of PO (the least soluble compound) decreased. Furthermore, the selectivity of SQ clearly reduced in the quaternary system. Raising the pressure the solubility of PO increased, causing a decrease in the concentration of both SQ and OA in the gaseous phase. The results indicate that the presence of small concentrations of OA in the feed lead to selectivity enhancement of SQ than that with higher concentrations of OA. Selectivity of SQ in the ternary and quaternary systems as function of pressure is shown in Fig. 8. Similar trends have been reported in

Table 6
Solubility data of SQ and PO in binary systems (SQ/PO + CO₂) and ternary systems (SQ + PO + CO₂) in SC-CO₂ at 313.15 K.

<i>P</i> (MPa)	SQ (<i>y</i> × 10 ⁵)		PO (<i>y</i> × 10 ⁵)		<i>α</i> (<i>y</i> _{SQ} / <i>y</i> _{PO})
	SQ + CO ₂	SQ + PO + CO ₂	PO + CO ₂	SQ + PO + CO ₂	
10	35.35 ± 0.07	7.553 ± 0.09	2.426 ± 0.23	0.327 ± 0.25	23.1
15	83.69 ± 0.12	8.143 ± 0.21	3.941 ± 0.09	3.535 ± 0.38	2.30
20	113.0 ± 0.03	9.956 ± 0.15	5.779 ± 0.02	11.29 ± 0.14	0.88
25	145.3 ± 0.07	12.96 ± 0.11	12.01 ± 0.17	15.14 ± 0.27	0.86

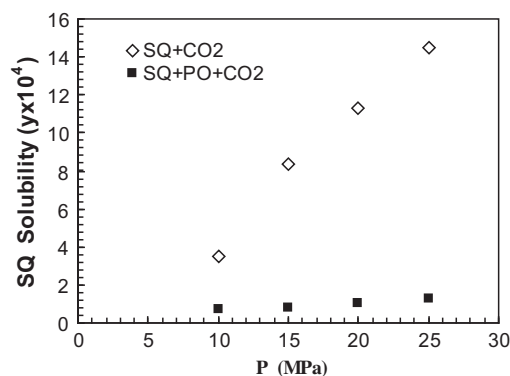


Fig. 6. Solubility isotherms of SQ at 313 K for the binary (SQ + CO₂) and ternary system (SQ + PO + CO₂).

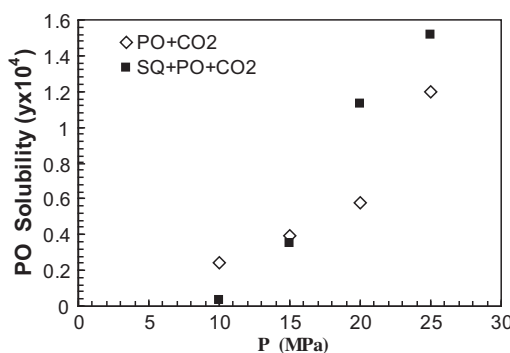


Fig. 7. Solubility isotherms of PO at 313 K for the binary (SQ + CO₂) and ternary system (SQ + PO + CO₂).

the literature for different lipid systems. Where the presence of FFA is studied, FFA is enriched in the vapour phase resulting in selectivity of SQ being decreased, which indicates poor separation between OA and SQ [24,25]. In order to investigate the distribution of lipid components in both gaseous and liquid phases, the distribution coefficient *K_i* of each component was calculated using following equation:

$$K_i = \frac{y_i}{x_i} \tag{3}$$

Here *y_i* is the mass fraction of component *i* in the gaseous phase and *x_i* is the mass fraction in the liquid phase. Distribution coefficients of SQ in the ternary system and quaternary system are presented in Fig. 9. Both SQ and FFA distribution coefficients were found to be higher than 1. SQ was enriched in the gaseous phase, reaching its maximum value at lower pressure, and decreasing with increasing pressure. It is also clear that SQ was more enriched than OA, as shown in Fig. 10. In contrast, PO was enriched in the liquid phase.

Table 7
Solubility data of SQ, PO and OA in ternary systems (SQ + PO + CO₂) and quaternary systems (SQ + PO + OA + CO₂) in SC-CO₂ at 313.15 K.

P (MPa)	SQ ($y \times 10^5$)		PO ($y \times 10^5$)		OA ($y \times 10^5$)	α		
	SQ + PO + CO ₂	SQ + PO + OA + CO ₂	SQ + PO + CO ₂	SQ + PO + OA + CO ₂	SQ + PO + OA + CO ₂	y_{SQ}/y_{PO}	y_{OA}/y_{SQ}	y_{OA}/y_{PO}
10	7.553 ± 0.09	4.063 ± 0.03	0.327 ± 0.25	0.850 ± 0.37	5.683 ± 0.19	4.78	1.40	6.69
15	8.143 ± 0.21	10.01 ± 0.13	3.535 ± 0.38	2.494 ± 0.48	24.59 ± 0.11	4.02	2.45	9.86
20	9.956 ± 0.15	12.12 ± 0.05	11.29 ± 0.14	7.701 ± 0.13	29.90 ± 0.06	1.57	2.46	3.88
25	12.96 ± 0.11	12.99 ± 0.13	15.14 ± 0.27	10.23 ± 0.04	32.11 ± 0.10	1.27	2.47	3.14

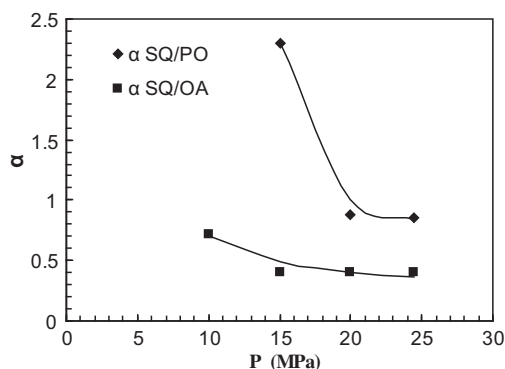


Fig. 8. Selectivity of squalene in SQ + PO mixture and SQ + OA + PO mixture as a function of pressure at 313 K.

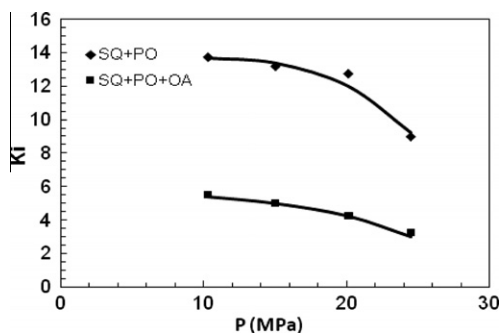


Fig. 9. Distribution coefficients of SQ in SQ + PO + CO₂ system and SQ + OA + PO + CO₂ system as function of pressure at 313 K.

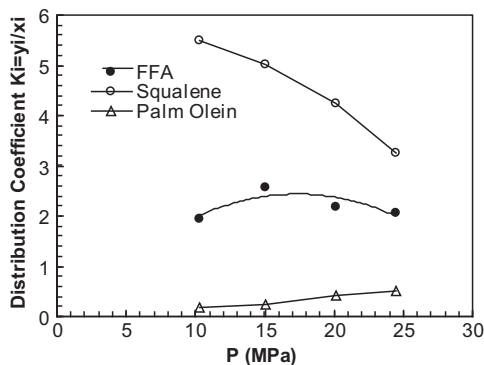


Fig. 10. Distribution coefficients of SQ, FFA and PO as function of pressure at 313 K.

4. Conclusions

Squalene has been shown to have a higher solubility than the other lipid components in the binary systems studied due to its

hydrocarbon and non-polar nature. Solubility investigation for the ternary and quaternary systems demonstrates the high selectivity of carbon dioxide towards squalene and free fatty acids. The presence of less soluble components, such as palm olein, affects the loading of the whole mixture, especially at a high concentration. The introduction of FFA into the SQ/PO mixture results in a decrease of carbon dioxide selectivity towards squalene. This is likely because the solubility of FFA is higher than the solubility of palm olein in CO₂, which reduces squalene vapour pressure, resulting in low selectivity. Solute-solute interactions have also been shown to affect the solubility in SC-CO₂. In conclusion this work demonstrates the feasibility of separating squalene and FFA from palm olein, by using the SC-CO₂ fractionation process.

Acknowledgements

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