Solubility of a Multicomponent Glyceride Mixture in SC-CO₂: Experimental Determination and Correlation

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ABSTRACT

Lipid compounds are gaining increasing attention due to a wide range of applications in the food industry. Particularly, supercritical carbon dioxide (SCCO₂) is being successfully employed as a green solvent in the extraction and fractionation of nonpolar lipids. Knowledge of the solubility of pure lipid-type substances and their mixtures in SCCO₂ is essential for evaluating the viability of supercritical separation processes and for their subsequent design. Hence, both solubility measurements and thermodynamic modeling are of interest to researchers and process engineers and are receiving considerable attention in the literature.
Solubility data of pure mono-, di- or triglycerides in SCCO₂ are to a certain extent reported in the literature. However, the information regarding solubility of complex multicomponent glyceride mixtures is relatively scarce.
In this work, we report the solubility of a commercial glyceride mixture (51.9 % wt monoglycerides, 34.0 % wt diglycerides and 14.1 % wt triglycerides) in SC-CO₂, measured in a variable-volume equilibrium cell at T = (313 and 323) K and pressures in the range of (10-35) MPa. Additionally, we apply two different equation of state based models, to represent the solubility data reported. The thermodynamic modeling is based on an appropriate selection of model substances to represent the glyceride mixture composition, which was determined by HPLC-ELSD and GC analysis, and an accurate estimation of the pure compounds properties required for the modeling.

INTRODUCTION

Monoglycerides and diglycerides are potentially attractive starting materials for numerous synthetic applications [1-4]. They can be employed for the synthesis of defined triglycerides, phospholipids, glycolipids, lipoproteins and preparation of numerous enzyme agonists and antagonists. Monoglycerides are the most commonly used surface-active lipids, and can be used for emulsification, aeration, as defoaming agents, oil stabilizers, etc. [3]. Additionally, diglycerides have been developed in recent years as substitutes for fat/oil [4].
Mono- and diglycerides are commonly produced by glycerol esterification with fatty acids, transesterification of triglycerides, or by lipase-catalyzed alcoholysis of oils. Usually, a mixture of mono-, di- and triglycerides is produced and purification of the reaction product is necessary. Potential new refining procedures are based on supercritical fluid technology [5]. Supercritical carbon dioxide (SCCO₂) has been widely studied as a potential solvent for nonpolar lipid extraction and fractionation. SCCO₂ is particularly useful when the lipid compounds have significant differences in molecular weight or vapor pressure.
Solubility data of pure mono-, di- and triglycerides in SCCO₂ is available in the literature [6-8]. Nevertheless, this information is scarce in the case complex multicomponent glyceride mixtures.
The solubility of monolaurin in SCCO₂ was found to be around 20 times lower than the solubility of di- and trilaurin [6]. Although trilaurin has a molecular weight 2.3 times higher and a vapor pressure 150 times smaller than monolaurin, the high polarity of monolaurin was found to limit its solubility in SCCO₂ [7]. When the fatty-acid chain length is increased, a greater monoglyceride solubility is obtained compared to the triglyceride solubility of an equivalent fatty-acid chain length, as demonstrated for the case of the monoolein/triolein pair [8]. Therefore, as the fatty-acid chain length increases, the difference in molecular weight and vapor pressure between the mono-, di- and triglyceride becomes more significant, minimizing the polarity differences of the respective lipid species.
In this work, the solubility of a commercial glyceride mixture (51.9 % wt mono-, 34.0 %wt di- and 14.1 % wt triglycerides) was measured in a variable-volume equilibrium cell at 313 and 323 K and pressures in the range of 10-35 MPa. Additionally, two different thermodynamic models, the predictive Group Contribution EoS and the SRK-EoS, were applied to represent the solubility data reported. The thermodynamic modeling is based on an

appropriate selection of model substances to represent the glyceride mixture composition, which was determined by HPLC-ELSD and GC analysis.

MATERIALS AND METHODS

Chemicals

The standards, 1-oleoyl-rac-glycerol, dioleoylglycerol and glyceryl trioleate were purchased from Sigma (St. Louis, MO). The sample mixture studied was from Sigma (St. Louis, MO) and is specified as a glyceride mixture which is high concentrated in rac-1-oleoyl-glycerol (monoolein). According to supplier the sample mixture may also contain (20-40) % of diglycerides and (20-40) % of triglycerides (TLC analysis). Trimethyl-2,2,4 pentane, methyl-t-butyl-ether, propan-2-ol and chloroform were of HPLC grade (Lab Scan, Dublin). Both, standards and samples were dissolved in chloroform prior analysis.

Phase equilibria equipment and measurement procedure

Experimental measurements were carried out by the static analytical method, using a high pressure variable volume cell. The equipment comprises a stainless cylinder vessel and a piston, which can be smoothly displaced inside the cylinder using a manual pressure generator. The cell is surrounded by a thick aluminum jacket, externally heated by two electrical resistances connected to a temperature controller ($\pm$ 0.1 K). A gauge transducer coupled with a digital indicator provides pressure measurements with an estimated accuracy of $\pm$ 0.2 bar. A detailed explanation of the experimental device can be found elsewhere [9].

Initially, a weighted amount of the glyceride mixture was loaded into the cell and it was purged with CO_2 to remove the residual air. Then, CO_2 was let to flow into the cell at ambient temperature until a pressure of 6.5 MPa was attained. Then, heating was turned on and once the equilibrium temperature was stabilized inside the cell, pressure was attained by means of the manual pressure generator. After stirring (1.5 h) the mixture was let to repose for 18 h in order that phase segregation occurred. A front window was employed to visually verify the existence of two phases.

Samples from top of the equilibrium cell were withdrawn using capillary lines and a micrometering valve, and decompressed to atmospheric pressure. Capillary lines and valve were thermostatized at the same temperature of the equilibrium cell. The manual pressure generator was employed to keep pressure constant during sampling ($\pm$ 1 bar). Glyceride samples were collected in glass vials and separated from the CO_2 by using a cool trap. Vials were weighed in a precision analytical balance (Ohaus Pionner PA214C) with 0.0001g accuracy. The amount of CO_2 was determined volumetrically using a 1000 ml graduated tube filled with a saturated solution of Na_2SO_4 to prevent CO_2 dissolution. The volume of gas was quantified as the displaced liquid volume and corrected by accounting for the effects of temperature, atmospheric pressure and water vapor pressure. All samples were collected by duplicate; deviations between the mass fractions obtained were lower than 0.025 %.

Chemical characterization

*HPLC-ELSD analysis***:** The glyceride mixture was dissolved in chloroform prior analysis, which was effected on a HPLC system from Varian (Middelburg, The Netherlands) equipped with two pumps (model Pro Star 210), an injector (model 500), a gradient module (Pro Star 500) and a ELSD PL-ELS 2100 Ice (Polymer Laboratories, Varian, Middelburg, The Netherlands). The column, ACE 5 SIL (250 mm x 4.6 mm, 5μm) was placed into an oven (Thar Instruments, Inc., Pittsburgh, PA) and maintained at 35ºC. The HPLC-ELSD method is based on that reported by Torres et al. [10] with some modifications. The solvent flow rate was 2 ml/min of (A) isooctane: MTBE (70:30) and (B) propan-2-ol using the gradient: 0 to 7 min, 100% (A); 8 to 15 min, 80% (A) and 16 to 20 min, 100% (A). The ELSD conditions were 1.6SLM nitrogen, 35ºC nebulizer, 35ºC vaporizer and gain 3.

GC analysis: Fatty acid methyl esters (FAME) were prepared by base catalyzed methanolysis of the glycerides according to ISO-IDF [11]. Analysis of FAME was performed on a gas-liquid chromatograph (Agilent 6890 N Network System, Agilent Technologies, Santa Clara, CA) onto a CP-Sil 88 fused silica capillary column (100 m $\times$ 0.25 mm, Varian, Middelburg, the Netherlands) under the conditions reported by Luna et al. [12]. The fatty acid esters were identified with a certified reference material from the European Community Bureau of Reference (CRM 164) and FAME standards from Nu-Chek Prep Inc. (GLC reference standards 455 and 461). C19:0 was used as internal standard.

Phase equilibria modeling

The Group Contribution Equation of State (GC-EoS): The residual Helmholtz energy is described by two terms [13]: a repulsive or free volume term and a contribution from attractive intermolecular forces. The free volume

contribution is modeled assuming hard sphere behavior for the molecules, and characterizing each substance i by a hard sphere temperature dependent diameter (d_i). For the evaluation of the attractive forces, a group contribution version of a density-dependent NRTL-type expression is derived. Interactions between like groups are given by their attraction energy parameter (g_{ii}) and interactions between unlike groups are calculated using mixing rules, which depend on the interaction energy parameter (k_{ij}) and the NRTL non-randomness parameter (α_{ij}). All pure component and group interaction parameters required to predict the phase equilibria behavior of the glycerides + CO_2 mixture studied in this work were recently reported in the literature [14]. A detailed description of the GC-EoS model equations is given in the work of Skjold-Jørgensen [13].

The SRK-EoS: The application of the SRK-EoS model [15] with the one-fluid van der Waals mixing rule to correlate the solubility of each of the three glycerides and the glyceride mixture in $SCCO_2$ requires knowledge of the critical parameters of the pure glycerides and the interaction parameters.

The critical properties of the pure glycerides, of interest to our study, are not experimentally accessible and should be considered as hypothetical quantities rather than properties with any physical meaning. Thus, they have to be estimated either by correlations or group contribution methods. In the present study, we employ the methods of Wakeham et al. [16] and Brauner et al. [17].

It should also be stressed that because there are no experimental data available with which to compare the estimated values, and because the values estimated by different authors for the three glycerides differ considerably, see for example [18, 19] to name just two of the most recently published studies, it is highly desirable to apply as an assessment tool of the reliability of the properties estimated the generalized semi-theoretical expression advocated by Zbogar et al. [20]:

$$T_c/P_c = 9.0673 + 0.43309(Q_w^{1.3} + Q_w^{1.95}) \tag{1}$$

where T_c is in Kelvin and P_c is in bar. The dimensionless parameter Q_w is a measure of the van der Waals molecular surface area and is calculated as the sum of the group area parameters, Q_k:

$$Q_w = \sum_k v_k Q_k \tag{2}$$

where v_k is the number of times group k appears in the molecule. The group area parameters Q_k are available in the UNIFAC tables.

RESULTS AND DISCUSSION

Experimental results
According to our analysis, the composition of the glyceride mixture (GM) studied in this work is: 51.9 % wt monoglycerides (monoolein), 34.0 % wt diglycerides and 14.1 % wt triglycerides.

The solubility of the GM in $SCCO_2$ at $T = (313$ and $323)$ K is reported in Table 1 and depicted in Figure 1 as a function of pressure. As expected, the solubility noticeably increases with pressure. Taking into account that the GM contains ca. 52 % of monoolein, solubilities measured in this work are in a reasonable agreement with previous data reported by Nilsson et al. for pure monoolein [8]. Also, considering the data at 323 K, it can be observed that the solubility of the GM mixture is slightly higher than that of pure monoolein.

Table 1. Solubility (% wt) of GM in $SCCO_2$.

T (K)	P (MPa)	Experimental	GC-EoS	SRK-EoS
313	10	0.25	0.01	0.17
	20	0.48	0.75	0.35
	30	0.69	1.26	0.62
	35	1.28	1.36	1.14
323	10	-	0.01	0.09
	20	0.68	0.50	0.52
	30	0.90	1.25	0.88
	35	1.38	1.46	1.25

The fatty acid ester composition of GM according to our analysis is: 3.9 % wt C8:0-C14:0; 4.1 % wt C16:0; 6.8 % wt C16:1; 1.2 % wt C17:0; 1.3 % wt C18:0; 79.0 % wt C18:1; 3.5 % wt C18:2; 0.2 % wt C18:3. That is, 84 % wt of the fatty acid esters detected are C18 fatty chains. Thus, the chemical composition selected by us in order to represent adequately the GM mixture was 51.9 % of monoolein, 34.0 % diolein, 14.1 % triolein.
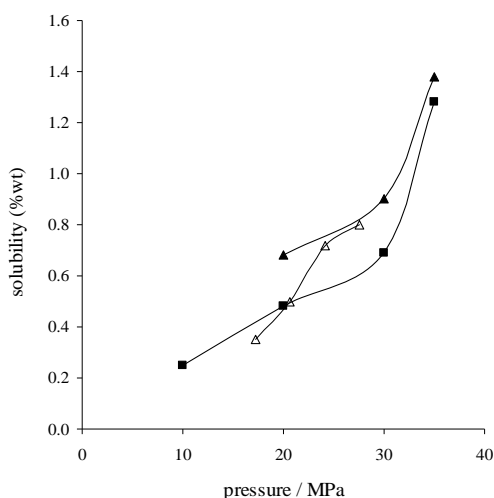

Figure 1. Solubility of the glyceride mixture (51.9 %wt monoolein) at (■) 313 K and (▲) 323 K. Comparison with the data reported by Nilsson et al. [8] for pure monoolein at (△) 323 K.

GC-EoS and SRK-EoS modeling
In order to test the applicability of the SRK-EoS to represent the solubility of the GM mixture in $SCCO_2$ firstly the critical parameters of pure mono-, di- and triolein were estimated applying [16, 17]. The values obtained are shown in Table 2. Our estimations give an excellent approximation to the theoretically calculated T_c/P_c ratio [20] for the three pure substances in contrast to those of [19]. The deviations of the latter are, in our view, due mainly to the too high values of the critical pressures predicted.
Then, the SRK EoS interaction and size parameters for each pair (glyceride + CO_2) were estimated from the best fit to the data measured by Nilsson et al. [8] applying a standard optimization procedure. The values obtained at T= 323 K are shown in Table 3.

Table 2. Critical parameters estimated for pure mono-, di- and triolein.

Glyceride	T_c (K)	P_c (bar)	omega	T_c/P_c [20]	T_c/P_c [this work]	T_c/P_c [19]
Mono-	858.35	9.13	1.2047	94.014	95.259	79.078
Di-	1005.92	4.41	1.3652	228.100	227.170	182.218
Tri-	1067.68	2.58	1.8751	413.030	415.688	292.778

Table 3. SRK-EoS interaction and size parameters (CO_2 - glyceride) at T = 323 K.

Component	k_{ij}	l_{ij}
Monoolein	0.084	-0.020
Diolein	0.105	-0.035
Triolein	0.117	-0.040

It should be noted that the k_{ij} values of Table 3 were employed also when correlating the solubility of the glyceride mixture in $SCCO_2$ at T = 313 K, owing to a lack of reliable experimental data for the glycerides solubility at that temperature. Thus, the interaction parameters were treated as non-temperature dependent.

Furthermore, the interaction parameters between the glycerides were set equal to zero at both temperatures, which is an additional rough approximation.

In the case of the GC-EoS model, all model parameters and pure component parameters were previously reported in the literature [11].

Figure 2 shows the quality of the GC-EoS (Figure 2a) and SRK-EoS (Figure 2b) predictions obtained in the calculation of the solubility of mono-, di- and triolein in SCCO$_2$ at 323K.

The agreement between the GC-EoS predictions and the experimental data is not good at lower pressures, but improves considerably in the range of $P = (20\text{-}30)$ MPa. Thus, the model can satisfactory differentiate between the solubility of the glycerides according to the number of fatty acid chains present in their corresponding molecular structure. Still, the main deficiency of the GC-EOS predictions remains the fact that it cannot represent correctly the trend of the solubility increase of diolein with pressure in comparison to the monoolein trend. One of the reasons could be the fact that the alcohol-ester interaction parameters of the GC-EoS model were determined from vapor-liquid equilibrium data available for (ester + alcohol) mixtures [21], i.e. no experimental data related to mono- or diglyceride + CO$_2$ mixtures was employed. The deviations between experimental and calculated mono-, di- and triolein solubilities:

$$StD\% = (100/N) \cdot \sqrt{\sum \left[\left(S_i^{\exp} - S_i^{cal} \right) / S_i^{\exp} \right]^2}$$

obtained with the GC-EoS model were, respectively, (7.7, 7.9 and 12.3) %.

The SRK-EoS, on the other hand, describes correctly the solubility trend of the three glycerides in SCCO$_2$ at $T = 323$ K (see Figure 2b). Still, the quantitative agreement is not very good. Furthermore, the SRK EoS solubility correlations don't represent a considerable improvement over the results obtained with the GC-EoS model, for which the parameters were regressed using equilibria data other than glyceride + CO$_2$ mixtures. A possible explanation can be sought in the fact that the SRK EoS interaction parameters were treated as non-temperature dependent and that those between the glycerides were set equal to zero.
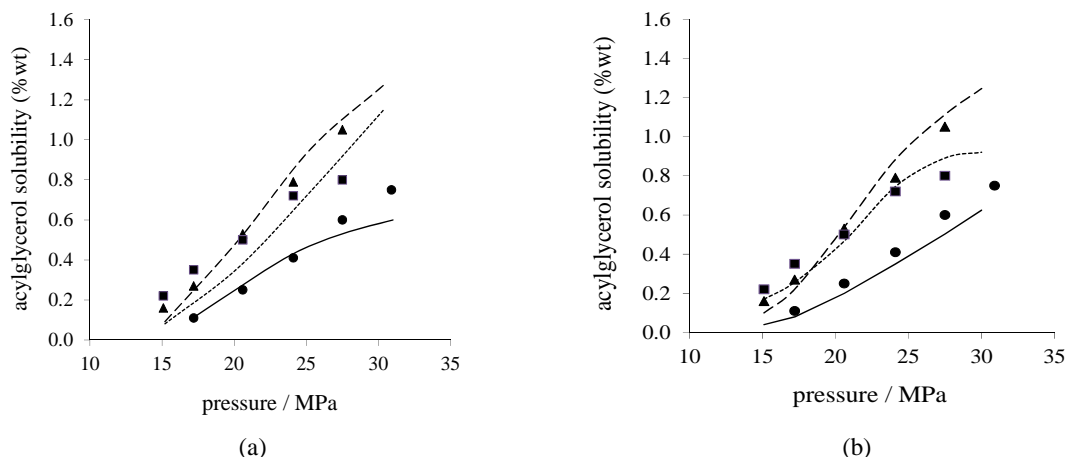

Figure 2. Glyceride solubility in SCCO$_2$ at 323 K. Experimental data [8]: (■) monoolein; (▲) diolein; (●) triolein. Model predictions: (-----) monoolein; (– – –) diolein; (——) triolein. (a) GC-EoS; (b) SRK-EoS.

The prediction of the GM solubility obtained with both thermodynamic models is given in Table 1. The *StD %* deviation were (6.8 and 3.6) % for, respectively, the GC-EoS and SRK models. Thus, both models were able to produce quite acceptable prediction (GC-EoS) and correlation (SRK) of the solubility of this complex mixture in SCCO$_2$.

CONCLUSIONS

The solubility of a commercial glyceride mixture in SCCO$_2$ was measured at (313 and 323) K and pressures up to 35 MPa. The appropriate definition of the model substances selected to represent its composition, determined

by HPLC-ELSD and GC analysis, allowed the application of a completely predictive (GC-EoS) and a semi-empirical (SRK EoS) to model its solubility. The results obtained demonstrated that both models can be used with confidence to predict the solubility of pure lipid-type substances and their mixtures in SCCO$_2$ which is essential for evaluating the viability and subsequent design of separation processes of interest to the food industry.

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