RESEARCH NOTE

SUPERCRITICAL FLUID EXTRACTION OF CAROTENOID FROM MICROALGAE WITH PROJECTED THERMODYNAMIC MODELS

R. Davarnejad, K. M. Kassim*, A. Zainal and S. A. Sata

School of Chemical Engineering, Engineering Campus, University Science Malaysia
14300 Nibong Tebal, Penang, Malaysia
davarnejad.usm.ac@gmail.com - chqassim@eng.usm.my - chzahmad@eng.usm.my

*Corresponding Author

Abstract   In this study, two thermodynamic models (regular solution theory and equation of state) were applied to obtain carotenoid solubility in the supercritical carbon dioxide solvent. Theoretical data obtained from the models were compared with the experimental data extracted from a published paper. The use of equation of state as an empirical correlation for collating and predicting liquid-liquid and liquid-dense fluid equilibrium is discussed. It is concluded that the estimation of some parameters required for these calculations would be difficult if the solute (carotenoid) was a complex substance in which little information was known, apart from its structural formula. An alternative procedure is to apply activity coefficient expression of the regular solution equations for each phase. Calculations along these lines are described and the physical basis for applying these methods under the relevant conditions was also presented. The regular solution theory approach in particular has been found to be encouraging for the mutual miscibility calculations for heavy components (such as carotenoid) particularly substances that are sensitive to temperature, though the interaction parameters for the prediction activity coefficients must be regarded as pressure dependent.

Keywords   Activity Coefficient, Carotenoid, Equation of State, Prediction, Regular Solution Theory, Solubility

چکیده   در این مقاله، دو مدل ترمو دینامیکی شامل تئوری محلول های با قاعده و معادله حالت بکار گرفته شد تا حلالیت کارتن در حلال فوق بحرانی دی اکسید کربن بدست آورده شود. دیتاهای حاصل از مدل ها با دیتاهای آزمایشگاهی منتشر شده در یک مقاله مقایسه شدند. کاربرد معادله حالت به عنوان یک تطبیق تجربی برای مطابقت سازی و پیشگویی تعادل سیال مایع – مایع و بخار – مایع بحث شد. نتیجه گیری شد که تخمین بعضی از پارامترهای خواسته شده برای این محاسبات مشکل خواهد بود در صورتی که ماده حل شونده (کارتن) یک جزء پیچیده باشد که تنها اطلاعات کمی از فرمول ساختاری آن شناخته شده باشد. یک روند جایگزین بر مبنای ضریب فعالیت معادلات محلول های با قاعده برای هر فاز بکار گرفته شد. محاسبات در طول این مسیرها شرح داده می‌شوند و پایه های فیزیکی برای بکار گیری ای روش ها تحت شرایط مربوط همچنین معرفی می‌شوند. معبر تئوری محلول های با قاعده در این خصوص به طور ترغیب آمیزی با محاسبات حلالیت ترکیبات سنگین (شبیه کارتن) خصوصاً آن دسته از موادی که به دما حساس بوده اند فهمیده شده است. اگر چه پارامترهای اثر متقابل بر پیشگویی ضرایب فعالیت بایستی به عنوان عامل وابسته به فشار مورد توجه واقع شوند.

1. INTRODUCTION

Conventional methods, based on the solvent extraction from natural matrices are time consuming. This method requires multiple extraction steps and large amounts of organic solvent, which are often expensive and potentially harmful. Extraction with carbon dioxide under supercritical conditions constitutes as an emerging technology in terms of reducing environmental impact. The advantages of using this technology are its lack of toxicity, chemical inertness, critical temperature

and pressure available, low cost and availability [1]. Furthermore, the use of carbon dioxide is also beneficial in adding quality to the products obtained since this technique does not give rise to excessive heating which usually has a negative effect on the heat-sensitive compounds [2].

Carotenoids are increasingly used in food technology mainly due to consumer pressure and more demanding regulations regarding the use of artificial dyes [2]. Carotene is a precursor of vitamin A in human and animal metabolism and it is used in the food processing industry for coloring purposes. Carotene has a large aliphatic molecule and one of its isomers, β-carotene, is illustrated in Figure 1 [3].

Supercritical CO_2 has a low solubility for β-carotene (and other pigments) most of which is concentrated in the raffinate [4,5]. System pressure was found to be more significant rather than temperature for increasing the solubility in supercritical CO_2 [4]. Some researchers have predicted the solubility of some bio-molecules such as β-carotene and cholesterol by using the equations of state [6]. The objective of this study is to predict mutual solubility for a system involving carbon dioxide and carotene as heavy component with supercritical carbon dioxide solvent. Theoretical data were calculated from regular solution equations and compared with the experimental data. These equations are described in detail by King, et al [7]. Calculations using the proposed equation mentioned above are defined and described in this paper, together with the physical basis for applying the proposed methods under the relevant conditions. Some of the interaction parameters that are required for the calculation of activity coefficients can be calculated from the experimental data for some equilibrium systems which have been mentioned in references [8,9]. The other interaction parameters have been generated by Fredenslund, et al [10].
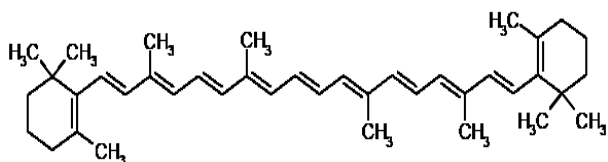

Figure 1. Structure formula of β-carotene.

The obtained data, activity coefficient, Gibbs function relationships and eventually mutual solubility data are calculated for two equilibrated phases by using regular solution equations.

2. REGULAR SOLUTION THEORY

The regular solution theory adopted as a model for this system is based on the activity coefficients by applying the following equations:

$$RT\ln\gamma_i = \left(\frac{d\left(nG_{mixing}^{Excess} \right)}{dn_i} \right)_{T,P,n_{j \neq i}} =$$

$$\left(\frac{d\left(nH_{mixing}^{Excess} \right)}{dn_i} \right)_{T,P,n_{j \neq i}} - \quad (1)$$

$$T\left(\frac{d\left(nS_{mixing}^{Excess} \right)}{dn_i} \right)_{T,P,n_{j \neq i}}$$

$$\ln \gamma_i = (\ln \gamma_i)^{Extract} + (\ln \gamma_i)^{Solute} \quad (2)$$

$$(\ln\gamma_i)^{Extract} = \frac{1}{R \times T} \times \left(\frac{d\left(nH_{mixing}^{Excess} \right)}{dn_i} \right)_{T,P,n_{j \neq i}} =$$

$$\left(\frac{\varphi_j^2 \times V_{mi}}{R \times T} \right)\left(d_i - \zeta_{ij}d_j\right)^2 + 2l_{ij}d_id_j\zeta_{ij}$$

$$(3)$$

Where

$$\zeta_{ij} = \left[\left(\frac{V_{mj}}{V_{mi}} \right)\left(\frac{q_i}{q_j} \right) \right]^{\frac{1}{2}} \quad (4)$$

and

$$d_i = \left[\frac{\left(U^0_{mi} - U_{mi} \right)}{V_{mi}} \right]^{\frac{1}{2}} \qquad (5)$$

d_i is the well known "solubility parameter" of component (i). U_{mi} and U^0_{mi} are the molar internal energy of the compressed fluid component (i) and the same fluid at the same temperature but a very low pressure. These parameters are calculated by the same equations but for component (j), as well. Equation 3 may be compared with the expression given by the Van der Waals approach [11],

$$(\ln\gamma_i)^{Extract} = \left(\frac{\varphi_j^{v2} \times V_{mi}}{R \times T} \right) \left(\left(d_i - d_j \right)^2 + 2l_{ij}d_id_j \right) \qquad (6)$$

It differs from this, only in the terms of ζ (which is usually close to unity) and in the replacement of the area function (φ_j^v) by the volume function,

$$\varphi_j^v = \frac{\left(x_j \times V_{mj} \right)}{\left(x_i \times V_{mi} + x_j \times V_{mj} \right)} \qquad (7)$$

Where (V_{mi}) is the molar volume of the pure liquid (i).

For a non-spherical molecule of type (i), quantity (q_i) is defined such that (Zq_i) is the number of interactions made by a molecule of this type with surrounding molecules. A monomer has (Z) interactions with nearest neighbor molecules (following X-ray diffraction information for simple fluids Z is normally given a value of 10). (q_i) is termed the area function for the molecule. For a linear molecule [12,13],

$$q_i = r_i - \left(\frac{2 \times (r_i - 1)}{Z} \right) \qquad (8)$$

(r) is the number of segments and it is calculated as a function of number of carbon atoms, for example (n) for alkanes can be determined for the calculation, therefore,

$$r = 0.90 + 0.283 \ (n{-}1)$$

Also, there would be an arrangement for Equation 3 as following,

$$\ln\gamma_i^{Extract} = \Sigma_{K=1}^{N} v_K^{(i)} \left(\ln\Gamma_K - \ln\Gamma_K^{(i)} \right) \qquad (9)$$

$$\ln\Gamma_K = Q_K \left[1 - \ln\left(\Sigma_m^N \theta_m \Psi_{mK} \right) - \sum_{m=1}^{N} \frac{\theta_m \Psi_{Km}}{\Sigma_{n=1}^N \theta_n \Psi_{nm}} \right] \qquad (10)$$

Where

$$\Psi_{mK} = Exp\left(-\frac{a_{mn}}{T} \right) \qquad (11)$$

Q_k is the area function for group k and θ_m is the area fraction of group m. $\ln\Gamma_k^{(i)}$ is defined similarly except that the group area fractions refer to the pure liquid i and not to the mixture.

$$(\ln\gamma_i)^{Solute} = \ln\left(\frac{\varphi_i}{x_i} \right) + \left(\frac{Zq_i}{2} \right) \times \ln(\theta_i/\varphi_i) + l_i - (\varphi_i/x_i) \sum_{j=1}^{M} x_j l_j \qquad (12)$$

Where M, θ_i and ϕ_i are the number of components in the solution, the area fraction for component i in the solution and the segment fraction, respectively.

$$l_i = (Z/2)(r_i - q_i) - (r_i - 1)$$

$$\varphi_i = \frac{x_i r_i}{\sum_{i=1}^{M} x_i r_i}$$

Also there is another arrangement for Equation 12,

$$(\ln\gamma_i)^{Solute} = \ln\left(\frac{\varphi_i}{x_i} \right) - \left(\frac{Zq_i}{2} \right) \times \ln\left[1 + \left(2\varphi_j/Zq_i \right) \left(\left(r_i/r_j \right) - 1 \right) \right] \qquad (13)$$

Which are a function of interaction parameters and zero entropy.

In the present work, activity coefficient is calculated using Equations 2, 9 and 12.

In order to present the calculations detail of the mutual solubility for the system CO_2 (i)/heavy component (j) it is necessary to define that x_i^E is the mole fraction of component (i) based on the extract phase (carbon dioxide) and x_i^S is the mole fraction of component (i) based on the solute phase. Therefore x_i^E and x_i^S can be calculated from the activity coefficients data γ_i^E and γ_i^S for the phases and from the distribution factors k_i and k_j as:

$$\gamma_i^E x_i^E = \gamma_i^S x_i^S \tag{14}$$

$$k_i = \frac{x_i^E}{x_i^S} \tag{15}$$

The procedure shown as followed:

a. Guessing initial k-values for each component given by Equation 15.
b. Use these guessed k-values to obtain the approximate mole fraction of component (i) in each layer.

$$x_i^E = \frac{1 - k_j}{1 - \frac{k_j}{k_i}} \tag{16}$$

$$x_i^S = \frac{x_i^E}{k_i} \tag{17}$$

These values were then inserted at step b and the cycle was repeated until the mole fractions calculated in step b showed negligible change from one step to the next.

An alternative approach which was used in the regular solution theory calculations was to establish analytic expressions for a function Q and its derivatives with respect to mole fraction given by:

$$Q_i = -\left[x_i \ln(x_i \gamma_i) + x_j \ln(x_j \gamma_j) \right] = -\frac{G_m^{mixing}}{RT} \tag{18}$$

$$\left(\frac{\partial Q_i}{\partial x_i} \right)^E = \left(\frac{\partial Q_i}{\partial x_i} \right)^S = \frac{\left(Q_i^E - Q_i^S \right)}{\left(x_i^E - x_i^S \right)} \tag{19}$$

$$\frac{dQ_i}{dx_i} = -\ln \frac{\gamma_i x_i}{\gamma_j x_j} \tag{20}$$

$$\frac{d^2 Q_i}{dx_i^2} = -\frac{d\left(\ln \frac{\gamma_i x_i}{\gamma_j x_j} \right)}{dx_i} \tag{21}$$

(G_m^{mixing}) is the molar Gibbs function of mixing and from standard thermodynamic relationships, it follows that $(\partial^2 Q_i / \partial x_i^2)$ should be negative at all points in a completely miscible system. If the system is partially miscible there will be a region over which $(\partial^2 Q_i / \partial x_i^2)$ is positive. In the latter case the points on the Q_i versus x curve corresponding to the equilibrium phase extract (E) and solute (S) have a common tangent Equation 19, where $(\partial^2 Q_i / \partial x_i^2)^E$ = gradient, $(\partial^2 Q_i / \partial x_i^2)^E$ taken at the mole fraction (x_i^E) of component (i) in the solvent-rich phase and $(\partial^2 Q_i / \partial x_i^2)^S$ = gradient taken at mole fraction (x_i^S) of component (i) in the solute-rich phase.

If the first assumption is good as (estimations of the values of $(x_i^E)^0$ and $(x_i^S)^0$) for the mole fractions (x_i^E) and (x_i^S)) which are already available, the applied procedure was found to be satisfactory for locating (x_i^E) and (x_i^S) using Equation 19.

This procedure was repeated until no further adjustment was required. Equation 19 was then satisfied and the mole fractions (x_i^E) and (x_i^S) specified the required calculated phase compositions.

3. RESULT AND DISCUSSION

By using the liquid-liquid equilibrium data for the system carbon dioxide-hexadecane at required pressures and at temperature of 25°C [8,9], as shown in Figure 2, together with the regular solution derived model [7,14,15] in order to calculate effective values of the $a_{CO2/CH \text{ (or CH2 or CH3)}}$
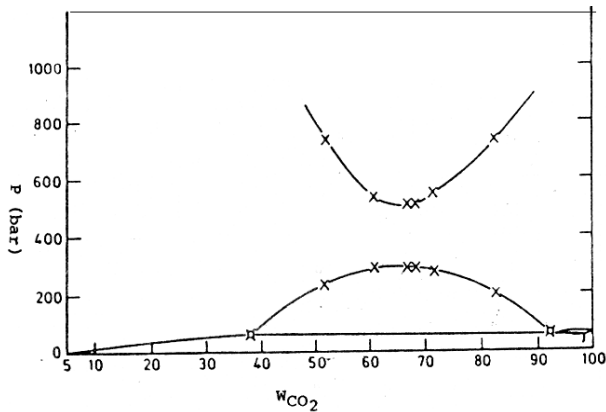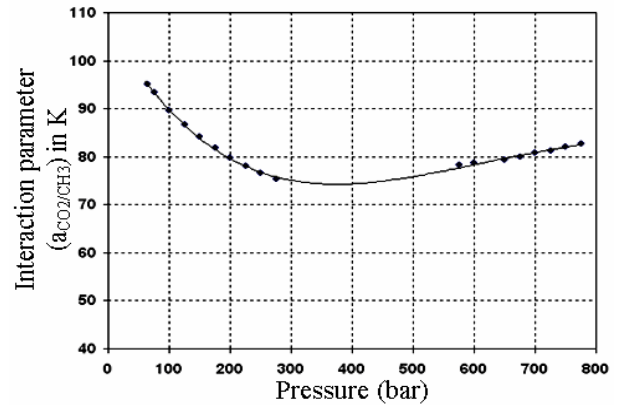
Figure 2. Pressure-composition diagram for system carbon dioxide/n-hexadecane at 25°C and at pressure up to 1000.0 bars. Upper critical solution pressure of about 300 bars. Lower critical solution pressure of about 500 bars. ×: Interpolated from [8]. ○: Interpolated from [9].
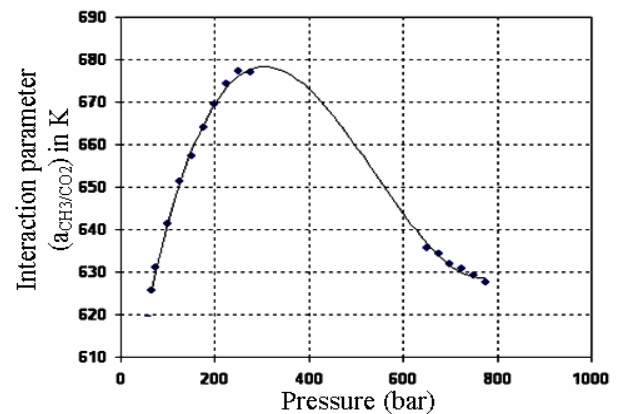

Figure 3. Regular solution theory parameter ($a_{CO2/CH3}$) interactions as function of pressure at 25°C. These data obtained from the experimental data for the CO_2/n-hexadecan system as shown in Figure 2 by applying the regular solution equations.

and $a_{CH/CO2}$ interactions as a function of pressure as shown in Figures 3 and 4. These data used to predict mutual solubility of the carbon dioxide-carotenoid system at temperatures of 40, 50, 60°C and at pressure up to 500 bar. Parameters other than $a_{CO2/CH2}$ and $a_{CH2/CO2}$ required in the regular solution model were evaluated using the standard methods described in [10].

In order to examine the behavior of the system of CO_2-carotenoid at various pressures and temperatures, miscible or immiscible, it is necessary to calculate the values of the activity coefficient (γ), Gibbs function (G) and its derivatives (dG/dx) and (d^2G/dx^2), using Equations 2, 9, 12, 18, 20 and 21.

Then the experimental data were extracted from reference [2]. Table 1 shows the ratio (the yield of extracted carotene per yield of extracted chlorophyll a) at different temperatures and at various pressures while in Table 2 it shows the yield of extracted carotene (µg carotene/ mg dry weight microalgae, i.e. feed) and carotene mole fractions (in extracted phase) related to temperature and pressure. By applying the experimental data extracted from Figure 2 and regular solution equations, the interaction parameters as function of pressure and at 25°C were obtained. The results were ploted in Figures 3 and 4. Interaction parameters were obtained at

Figure 4. Regular solution theory parameter ($a_{CH3/CO2}$) interactions as function of pressure at 25°C. These data obtained from the experimental data for the CO_2/n-hexadecan system as shown in Figure 2 by applying the regular solution equations.

required pressures and it was tabulated and shown in Table 3.

Figure 5 shows the CO_2 activity coefficient (γ_{CO2}) calculated from Equations 2, 9 and 12 against CO_2 mole fraction (x_{CO2}) at temperature of 40°C and at variable pressures. Interaction parameters $a_{CH3/CO2}$ and $a_{CO2/CH3}$ are obtained from Table 3. Furthermore, Figures 6 and 7 show the CO_2 activity coefficient (γ_{CO2}) at temperatures of

TABLE 1. Pressure/Yield/Ratio of Carotenoid at 40, 50 and 60°C [2].

T = 40°C			T = 50°C			T = 60°C		
P (bar)	Yield	Ratio	P (bar)	Yield	Ratio	P (bar)	Yield	Ratio
200	0.152	0.524	200	0.152	0.410	200	0.125	1.389
300	0.208	0.258	300	0.248	0.230	300	0.250	0.179
400	0.152	0.115	400	-	-	400	0.340	0.153
500	0.104	0.129	500	0.180	0.129	500	0.252	0.120

TABLE 2. Experimental Data Extracted from Table 1.

T = 40°C			T = 50°C		T = 60°C	
P (bar)	Yield	$(x_{carotene})^{Exp}$	Yield	$(x_{carotene})^{Exp}$	Yield	$(x_{carotene})^{Exp}$
200	0.152	1.24609×10^{-5}	0.152	1.25×10^{-5}	0.125	1.03×10^{-5}
300	0.208	0.0167680	0.248	2.04×10^{-5}	0.250	2.05×10^{-5}
400	0.125	1.0300×10^{-5}	-	-	0.340	2.79×10^{-5}
500	0.104	8.6000×10^{-6}	0.180	1.48×10^{-5}	0.252	2.07×10^{-5}

**TABLE 3. Interaction Parameters $a_{CH3/CO2}$ and $a_{CO2/CH3}$ Obtained from Figures 3 and 4 as Function of Pressure and at 25°C.**

CO$_2$-Hexadecane, T = 25°C				
P (bar)	$(x^{E}_{CO2})^{Exp}$	$(x^{S}_{CO2})^{Exp}$	$a_{CH3/CO2}$	$a_{CO2/CH3}$
200	0.960416	0.870833	669.5154	79.8003
300	0.910416	0.889583	620.1960	78.7003
400	-	-	673.8462	72.2857
500	-	-	658.4615	75.7143
550	0.927500	0.888542	624.8088	78.4003
600	0.939583	0.870416	634.1288	78.6003
700	0.956700	0.854167	631.7817	80.7003

50 and 60°C, respectively. These figures showed that activity coefficient for CO_2 decreases with increasing CO_2 mole fractions. These figures also showed that activity coefficient decreased from 200 bar to 400 and 500 bar and then 300 bar for a constant mole fraction of CO_2. It means that this system (carotenoid-CO_2) based on CO_2 compound is near the ideal solution at 300 bar, especially in CO_2 mole fractions close to one.

Experimental and theoretical data were
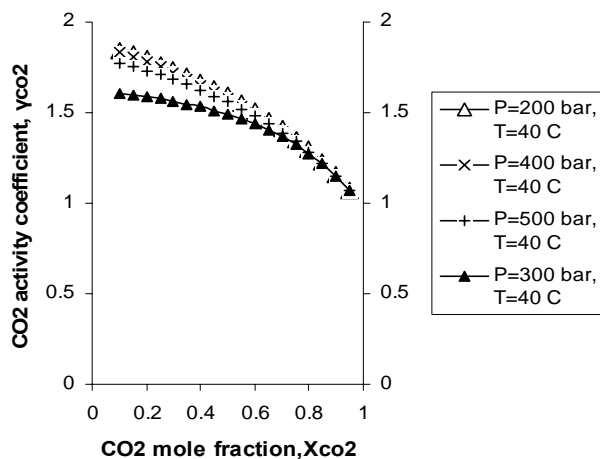
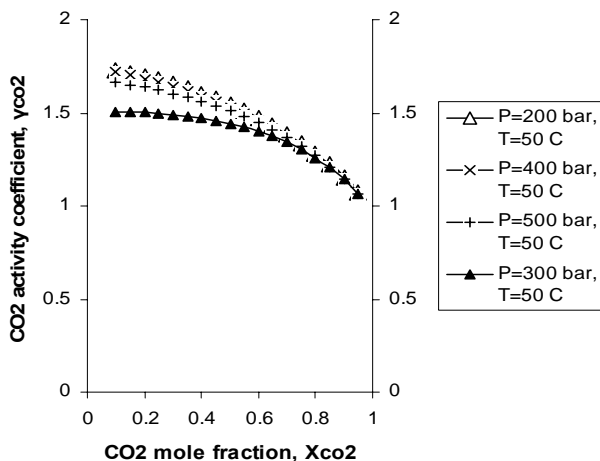**Figure 5**. CO_2 activity coefficient (γ_{CO_2}) against CO_2 mole fraction (x_{CO_2}) at constant temperature of 40˚C and at variable pressures.
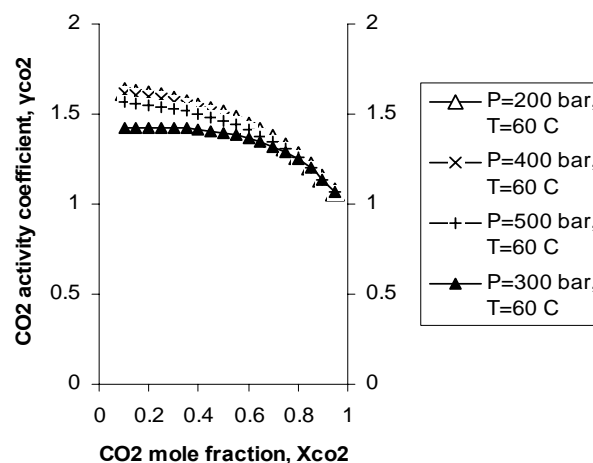
Figure 7. CO_2 activity coefficient (γ_{CO_2}) against CO_2 mole fraction (x_{CO_2}) at constant temperature of 60˚C and at variable pressures.

Figure 6. CO_2 activity coefficient (γ_{CO_2}) against CO_2 mole fraction (x_{CO_2}) at constant temperature of 50˚C and at variable pressures.

compared in Tables 4-6. They show the experimental and theoretical data based on CO_2 compound at 40, 50 and 60˚C and at variable pressures, respectively. The error percentage was calculated for the extracted phase data. The results showed that regular solution theory can predict carotenoid solubility in supercritical carbon dioxide well.

CO_2-carotenoid system was also modeled by applying Redlich-Kwong equation of state (RK).

All equations are shown in reference [16]. Some necessary parameters such as critical properties and RK constants are also shown in Table 7. The results according to RK equation of state for extracted phase at temperatures of 40, 50, 60 and at variable pressures were tabulated in Tables 8-10, respectively. The error percentage was calculated for the extracted phase data. The results showed that RK equation of state can predict carotenoid solubility in supercritical carbon dioxide very well.

4. CONCLUSION

This study predicted the solubility data of β-carotene at supercritical conditions using the regular solutions theory approach. The theoretical equations and associated parameters used in the modeling are clearly described. The β-carotene experimental data and Redlick-Kwong parameters are obtained from published literature.

In this study, two applied models could properly predict carotenoid solubility in supercritical carbon dioxide. It concluded that the regular solution theory as a general model can be applied for different systems and at various conditions. The significant difference between regular solution model and other models (such as

TABLE 4. Comparison of the Experimental and Theoretical (Obtained from the Regular Solution Equations) Data at 40°C and at Variable Pressures.

P (bar)	$(x^E_{CO2})^{Exp}$	$(x^E_{CO2})^{RST}$	$(x^S_{CO2})^{RST}$	$E^E\%$
200	0.999987500	0.9987	0.7188	0.128
300	0.983231902	0.9984	0.7610	1.542
400	0.999989752	0.9981	0.7394	0.189
500	0.999991473	0.9983	0.7402	0.169

TABLE 5. Comparison of the Experimental and Theoretical (Obtained from the Regular Solution Equations) Data at 50°C and at Variable Pressures.

P (bar)	$(x^E_{CO2})^{Exp}$	$(x^E_{CO2})^{RST}$	$(x^S_{CO2})^{RST}$	$E^E\%$
200	0.99998750	0.9984	0.7429	0.158
300	0.999979665	0.9981	0.7826	0.188
400	-	0.9977	0.7632	-
500	0.999985242	0.9980	0.7635	0.198

TABLE 6. Comparison of the Experimental and Theoretical (Obtained from the Regular Solution Equations) Data at 60°C and at Variable Pressures.

P (bar)	$(x^E_{CO2})^{Exp}$	$(x^E_{CO2})^{RST}$	$(x^S_{CO2})^{RST}$	$E^E\%$
200	0.999989752	0.9981	0.7652	0.189
300	0.999979501	0.9977	0.8025	0.228
400	0.999972120	0.9973	0.7850	0.267
500	0.999979337	0.9976	0.7850	0.238

TABLE 7. Critical Properties of β-Carotene, Carbon Dioxide and RK Constants [6].

Component / Parameters	CO_2	$C_{40}H_{56}$
P_c(bar)	73.82	8.09
T_c(K)	304.19	801
Ω_1	0.42748	0.42748
Ω_2	0.08664	0.08664

TABLE 8. Comparison of the Experimental and Theoretical (Obtained from RK Equation of State) Data at 40°C and at Variable Pressures.

P (bar)	$(x^E_{CO2})^{Exp}$	$(x^E_{CO2})^{RK}$	$a_i \times 10^{-7}$	b_i	$a_j \times 10^{-9}$	b_j	$a_{ij} \times 10^{-}$	k_{ij} [6]	E^E %
200	0.99998750	0.99999786	6.4599	29.6824	6.6324	713.2018	1.2522	-0.913	1.036×10^{-3}
300	0.98323190	0.99999929	6.4599	29.6824	6.6324	713.2018	1.2522	-0.913	1.705
400	0.99998975	0.99999957	6.4599	29.6824	6.6324	713.2018	1.2522	-0.913	9.820×10^{-4}
500	0.99999147	0.99999969	6.4599	29.6824	6.6324	713.2018	1.2522	-0.913	8.220×10^{-4}

TABLE 9. Comparison of the Experimental and Theoretical (Obtained from RK Equation of State) Data at 50°C and at Variable Pressures.

P (bar)	$(x^E_{CO2})^{Exp}$	$(x^E_{CO2})^{RK}$	$a_i \times 10^{-7}$	b_i	$a_j \times 10^{-9}$	b_j	$a_{ij} \times 10^{-8}$	k_{ij} [6]	E^E %
200	0.999987500	0.99999543	6.4599	29.6824	6.6324	713.2018	7.295	-0.1145	7.930×10^{-4}
300	0.999979665	0.99999508	6.4599	29.6824	6.6324	713.2018	7.295	-0.1145	1.542×10^{-3}
400	-	0.99999839	6.4599	29.6824	6.6324	713.2018	7.295	-0.1145	-
500	0.999985215	0.99999903	6.4599	29.6824	6.6324	713.2018	7.295	-0.1145	1.382×10^{-3}

TABLE 10. Comparison of the Experimental and Theoretical (Obtained from RK Equation of State) Data at 60°C and at Variable Pressures.

P (bar)	$(x^E_{CO2})^{Exp}$	$(x^E_{CO2})^{RK}$	$a_i \times 10^{-7}$	b_i	$a_j \times 10^{-9}$	b_j	$a_{ij} \times 10^{-8}$	k_{ij} [6]	E^E %
200	0.999989572	0.99996780	6.4599	29.6824	6.6324	713.2018	7.5012	-0.146	2.177×10^{-3}
300	0.999979501	0.99999392	6.4599	29.6824	6.6324	713.2018	7.5012	-0.146	1.442×10^{-3}
400	0.999972120	0.99999839	6.4599	29.6824	6.6324	713.2018	7.5012	-0.146	2.672×10^{-3}
500	0.999979337	0.99999906	6.4599	29.6824	6.6324	713.2018	7.5012	-0.146	1.972×10^{-3}

equations of state) is that the latter required critical constants for phase equilibrium data and therefore provide vapor phase only. But for regular solution model, the data obtained are totally related to group interaction parameters and independent of temperature. It is possible to predict liquid-liquid and vapor-liquid equilibrium from the knowledge of structural formula of the constituent molecular species. The system miscibility also can be obtained by applying the solubility parameter which was calculated from regular solution equations. In this study the interaction between the individual groups constituting the molecules was considered and group interaction parameters were generated together with parameters that described the size and shape of the molecules.

5. ACKNOWLEDGMENTS

6. NOMENCLATURE

Symbol	Description
RK	Redlich-Kwong equation of state
Exp.	Experimental data
RST	Regular solution theory
i	Solvent (carbon dioxide)
j	Solute (carotenoid)
x_i^E	Component i mole fraction in extracted phase
x_i^S	Component i mole fraction in liquid phase
Ratio	yield of carotene/yield of chlorophyll a
Yield	μg carotene/mg dry weight microalgae (feed)
E^E %	Error percentage based on the extracted phase mole fraction, E^E % = (Experimental data-Theoretical data)/Experimental data × 100
γ_i	Component i activity coefficient
$\partial^2 Q_i/\partial x_i^2$	Solubility parameter for component i

7. REFERENCES

1. Williams, J. R., Clifford, A. A. and Al-Saidi, S. H. R., "Supercritical Fluids and Their Applications in Biotechnology and Related Areas", *Molecular Biotechnology*, Vol. 22, (2002), 263-286.
2. Macánchez-Sánchez, M. D., Mantell, C., Rodríguez, M., Martínez de la Ossa, E., Lubián, L. M. and Montero, O., "Supercriticl Fluid Extraction of Caratenoids and Chlorophyll a from Nannochlopsis Gaditana", *J. Food Eng*., Vol. 66, (2005), 245-251.
3. Birtigh, A., Johannsen, M. and Brunner, G., "Supercritical Fluid Extraction of Oil-Palm Components", *J. Supercrit. Fluids*, Vol. 8, (1995), 46-50.
4. Markom, M., Singh, H. and Hasan, M., "Supercritical CO_2 Fractionation of Crude Palm Oil", *J. Supercrit. Fluids*, Vol. 20, (2001), 45-53.
5. Bhattacharjee, P., Singhal, R. S. and Tiwari, S. R. "Supercritical Carbon Dioxide Extraction of Cottonseed Oil", *J. Food Eng*., Vol. 79, (2007), 892-898
6. Hartono, R., Mansoori, G. A. and Suwono, A., "Prediction of Solubility of Bio-Molecules in Supercritical Solvents", *J. Chem. Eng. Sci*., Vol. 56, (2001), 6949-6958.
7. King, M. B., Kassim, K. M., Bott, T. R., Sheldon, J. R. and Mahmud, R. S., "Prediction of Mutual Solubilities of Heavy Components with Supercritical and Slightly Sub Critical Solvents: The Role of Equations of State and Some Applications of a Simple Expanded Lattice Model at Sub Critical Temperatures", *Ber. Bunsenges phys. Chem*., Vol. 88, (1984), 812-820.
8. Schneider, G., Alwani, Z., Horvath, E. and Frank, U., "Phase Equilibriums and Critical Phenomena in Binary Mixed Systems to 1500 Bars Carbon Dioxide with N-Octane, N-Undecane, N-Tridecane and N-Hexadecane", *Chem. Ing. Tech*., Vol. 39, (1967), 649-656.
9. Francis, A. W., "Ternary Systems of Liquid Carbon Dioxide", *J. phys. Chem*., (1954), 1058-1099.
10. Fredenslund, A., Gmehling, J. and Rasussen, P., "Vapor-Liquid Equilibria using Unifac", Elsevier Scientific Publication Company, Amesterdam-Oxford-New York, U.S.A., (1977).
11. Hildebrand, J. H., Prausnitz, J. M. and Scott, R. L., "Regular and Related Solutions", Van Nostrand-Reinhold Company, New York, U.S.A., (1970).
12. King, M. B., "Phase Equilibrium in Mixtures", Pergamon Press, U.K., (1969).
13. Guggenheim, E. A., "Mixtures", Oxford University Press, U.K., (1952).
14. Kassim, K. M. and Davarnejad, R., "Mutual Solubilities Prediction for Some Oils Derivatives with Supercritical and Slightly Sub-Critical Carbon Dioxide Solvent", *International Conference on Environment 2006*, Penang, Malaysia, (November 2006).
15. Kassim, K. M., Davarnejad, R. and Zainal, A., "Phase Equilibrium Studying for the Supercritical Fluid Extraction Process using Carbon Dioxide Solvent with 1.35 Mole Ratio of Octane to Ethanol Mixture", *Chem. Eng. J*., (October 1, 2007).
16. Kassim, K. M. and Davarnejad, R., "Supercritical Fluid Extraction of Binary Systems of Monoolein, Diolein and Triolein, using Carbon Dioxide Solvent", *Int. J. Chemical Reactor Engineering*, Vol. 5, (2007), 1-9.