

Solubility of Astaxanthin in Supercritical Carbon Dioxide and Extraction Efficiency of Astaxanthin Extraction from *H. pluvialis*

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INTRODUCTION

Astaxanthin is a type of carotenoids in form of antioxidant with about 500 times activity greater than vitamin E. A large amount of astaxanthin, around 0.5-5.0%, is available in *H. pluvialis* [1, 4]. Supercritical carbon dioxide (SC-CO₂) extraction has been reported to be effective for astaxanthin recovery with regarding to clean technology and environmental friendly perspective as it operates at relatively low temperature, and the separation of carbon dioxide from the extract can be carried out simply by depressurization [5]. A number of previously published literatures concerning astaxanthin extraction from *H. pluvialis* by SC-CO₂ are available [6, 7]. To make further development on the design of the extraction process, the information on astaxanthin solubility in SC-CO₂ is necessary.

Although there are a few reports on solubility of astaxanthin determination [8-9], inconsistencies among the solubility data were found. In this report, the solubility of astaxanthin in SC-CO₂ over the range of temperature and pressure of 40-80°C and 20-40 MPa was examined. Several solubility models, i.e. the density based Chrastil's model [10], the enhanced density based model [11] and the equation of state (EOS) model [12-16] were fitted to describe astaxanthin solubility in SC-CO₂ in the range of conditions employed.

MATERIALS AND METHODS

Procedure: The dynamic method was employed to determine the solubility of astaxanthin in SC-CO₂ using the experiment setup shown in Fig. 1. To carry out the

solubility measurement, 50 mg of astaxanthin standard (99.5%, ALEXIS[®] Biochemicals, USA) was loaded uniformly over 15 g of glass beads and then charged into a 10 ml stainless steel cylindrical reactor (Thar Tech., Inc., USA). It should be noted that the amount of astaxanthin standard (50 mg) was preliminarily determined to be sufficient for the experimental investigation of astaxanthin solubility in SC-CO₂ at all conditions employed. The reactor was then placed inside an oven used to control the temperature (40, 60 and 80°C) and back-pressure regulator, BPR (Akico co., Japan) used to control the pressure (20, 30 and 40 MPa). The CO₂ was allowed to flow through the system at slow enough flow rate (0.5 ml/min), preliminarily determined to be 0.5 ml/min, to ensure the system equilibration. The astaxanthin samples exiting the extraction vessel along with the flowing SC-CO₂ were collected at a 30 min time interval behind the back pressure regulator, where the effluent solute was trapped with 10 ml dichloromethane (99.5%, Wako Pure chemical Industries, Ltd, Japan) contained in a test tube placed in an ice bath to prevent degradation of the compound. The flow rate of gaseous CO₂ at atmospheric pressure (at 25°C), escaping from the solute during the experiment was measured by a wet gas meter (Shinagawa co., Japan).

The collected samples were analyzed for their astaxanthin contents using a High Performance Liquid Chromatography (HPLC). The accumulated molar amount of astaxanthin in the sample was plotted against the number of moles of carbon dioxide passed. The solubility in each condition was calculated from the initial slope of this curve in the unit of mole of astaxanthin per mole of carbon dioxide [17-18].

Sample Analysis: The HPLC analysis of the collected sample was carried out using evelosil C30 UG-5, 250×4.6 mm i.d. and 5.0-μm particle size column (Nomura Chem. Co., Japan). The mixed solvent composed of methanol:acetonitrile:triethylamine at 49.98:49.98:0.04% by volume was used as the mobile phase with an elution flow rate of 1.2 ml/min. The astaxanthin was detected at the wavelength of 480 nm. The solvent for trapping and HPLC analysis: dichloromethane (99.5%), as methanol (99.7%), acetonitrile (99.7%), triethylamine (99.0%) were obtained from Wako Pure chemical Industries, Ltd, Japan.

MODEL ESTIMATION

Types of solubility model:

Many types of solubility model were used to explain the solubility of substance in supercritical phase. This research selected the frequently used solubility models to correlate the astaxanthin solubility data. The models are described as follows:

Chrastil's model

Chrastil's model is the simplest and frequently used model based on the assumption that there is association between SC-CO₂ and solute molecules resulting in a formation of solvato-complex (XY_κ, X is solute molecule, Y is solvent molecule and κ is association number) at equilibrium condition. This simple model relates the solubility of solute (y, mole of astaxanthin/mole of CO₂) in SC-CO₂ to the density (ρ, g/L) of the solvent and absolute temperature (T, K) of the fluid as shown below:

$$- \tag{1}$$

α depends on heats of vaporization and solvation enthalpies of solute, which therefore indicates the influence of temperature changes inside the extraction vessel. Another constant, β, depends on the molecular weight of both CO₂ and solvent. [15].

Solubility model by Peng Robinson-Equation of State (PR-EOS)

This solubility determination model is more complex than the Chrastil's model as it involves the equation of state. The solubility model concerning the fugacity of components in dense gas is frequently employed [12-16]. This solubility model is conducted under the assumptions that: (i) supercritical phase is dense phase; (ii) the gas in the solid is almost negligible; (iii) gas phase is considered pure and one-component solid was considered pure solid phase; (iv) a solid mixture is considered to behave like a heavy liquid phase and there is interaction between solvent and solute.

This model expresses the solubility of solute in supercritical fluid as:

$$\text{---} \text{---} \text{---} \tag{2}$$

$\frac{p^{sat}}{P}$ is the solubility in the ideal gas where p^{sat} is the saturation (vapor) pressure of the pure solid. $\{^{sat}$ and ϕ are fugacity coefficient at saturation pressure p^{sat} and at system pressure P , respectively. Here, the solid molar volume, v_s , can be assumed to be pressure independent. Due to low vapor, the values of $\{^{sat}$ for most solutes can be taken as unity.

The fugacity of solid solute (ϕ) in fluid phase in Eq. (2) was estimated using PR-EOS (Peng-Robinson equation of state) defined in Eq. (3).

(3)

where v is the molar volume of the component, and a and b are model parameters, related to interaction parameter, k_{12} and adjustable parameter, c_{12} , due to different sizes of species, determined from quadratic mixing rules for binary mixture that was explained elsewhere [15].

The critical properties of pure component are estimated by Joback group contribution method as detailed in previous literatures [19-20].

Enhancement factor and the density based model

This model is designed for the determination of low volatile compounds with high molecular weight and absent properties data particularly at the critical condition Bartl et al., 1991 [10]. This model is derived based on the assumption that the system has the linear correlation between the enhancement factor and density as shown in Eq.

4. (4)

where $A' = A + B\rho_{\text{ref}}$, A' and B are constants at constant temperature, P_{ref} is a reference pressure conventionally taken as 1 bar and ρ_{ref} is reference density taken as 700 kg/m³.

Consideration of suitable solubility model:

The solubilities obtained from solubility models were compared with experimental data to determine the percent average absolute relative deviation (% AARD) between experimental and calculated (V_{cal}) solubility data using the following equation,

(5)

where n is the number of solubility data used in this evaluation. The model with the least %AARD was selected as the most suitable model for the solubility behavior.

RESULTS AND DISCUSSION

1. Dependency of astaxanthin solubility on pressure and temperature

Figure 2 demonstrates the effect of temperature and pressure in the range of 40 to 80°C and 20 to 40 MPa on the solubility of astaxanthin where the x-axis refers to the pressure of the system and the y-axis on the left hand side refers to solubility of astaxanthin in mole fraction of solute whereas the y-axis on the right hand side shows the astaxanthin solubility in dimension of gram of astaxanthin per 1 liter of solvent. This figure

demonstrates that the solubility of astaxanthin increased with increasing pressure. It is known that an increase in pressure enhances the density of CO₂ [15]. This enhances the interaction between solute and solvent leading to an increase in a higher level of dissolution. This is reflected in a higher solubility of astaxanthin in CO₂ as indicated in Eq. 2.

Figure 2 also demonstrates the effect of temperature on the solubility of astaxanthin in SC-CO₂ where an increase in temperature seemed to steadily raise the solubility. This result, however, indicates that CO₂ density is not the only factor that influence the solubility as increasing temperature resulted in a decreasing CO₂ density which should lower the solubility of astaxanthin. However, the solute vapor pressure was the other parameter that plays a significant role in this case. An increase in temperature led to a higher vapor pressure of astaxanthin which means that more astaxanthin was being extracted into the supercritical CO₂ phase, and this effect prevailed over that of CO₂ density which resulted in an increase in the solubility [6, 16].

2. Model Correlation

Comparison between solubility data of astaxanthin predicted by the three models mentioned above and experimental data are summarized elsewhere [21]. The results demonstrate that solubility data obtained by the equation of state can thoroughly and better fit with experimental data over the entire temperature and pressure ranges of 40-80°C and 20-40MPa as shown in Figure 3. This could be due to the fact that the equation of state model is the only model that takes into account the properties of solute at the critical condition such as vapor pressure, and the extraction conditions such as temperature and pressure, and this renders the model to be more versatile than the other two models. Therefore it is concluded here that the equation of state is the most suitable model for the estimate of solubility of astaxanthin as provided the minimum of %AARD, Eq. (5) as depicted in Table 1 which compares %AARD among these three models [21].

3. Comparison between solubility data and previous researches

Figure 4 demonstrates solubility data of astaxanthin standard obtained by the dynamic and static methods in this work and de la Fuente et al.'s research, respectively. In this figure, the x-axis is the solubility of astaxanthin and y-axis is the system pressure. One interesting observation from this plot is that the static method always gave lower

solubility than the dynamic method. The exact reason for this still could not be given from the evidence available at this present time, but it was believed to be due to the experimental technique. In static method as employed in [de la Fuente et al., 2006](#), the samples were taken intermittently from the extractor at a certain time period whilst a continuous sampling was employed in the dynamic method. This way, the change (drastic drop) in the pressure during the sampling period could adversely affect the solubility and a lower solubility could be observed. A dynamic method therefore is considered to provide a more accurate level of the solubility.

4. Evaluation of extraction efficiency of astaxanthin extraction from *H. pluvialis*

4.1 Without co-solvent: In this section, the evaluation of extraction efficiency using solubility data will be illustrated as a showcase using experimental extraction data from [Machmudah et al. \(2006\)](#) and [Krichnavaruk et al. \(2008\)](#) as a case study. [Figure 5](#) illustrates overall extraction curve of astaxanthin from standard astaxanthin (this work) and *H. pluvialis* [6] in range of pressures of 20–40 MPa at 70°C, x-axis is accumulative mole of used CO₂ and y-axis is accumulative mole of astaxanthin. The solubility could be calculated from the initial slope of this overall extraction curve which is based on the assumption that the initial of extraction is rich in investigated substance. Remark here that solubility refers to the amount of astaxanthin in the dimension of mole astaxanthin dissolves in 1 mole of CO₂. [Figure 5](#) demonstrates that the initial slope of astaxanthin from *H. pluvialis* at 40 MPa was greater than that at 20 MPa, which means that the largest amount of astaxanthin could dissolve in SC-CO₂ better at 40 MPa than at 20 MPa. To put it more simply, the solubility of astaxanthin is relatively high at 40 MPa. Moreover, [Figure 5](#) depicts that as a larger amount of CO₂ was applied, the slope was no longer a straight line, due primarily to the mass transfer limitation in astaxanthin extraction from *H. pluvialis*.

The effect of pressure on the solubility of astaxanthin in SC-CO₂ is demonstrated in [Figure 6](#). The symbols “+” and “o” represent the data from the extraction of astaxanthin from the cell of *H. pluvialis* which was obtained from the work of [Machmudah et al. \(2006\)](#) and [Krichnavaruk et al., 2008](#) whereas the different lines are the simulated solubility from the model which represents the data from the extraction of standard astaxanthin (from this work). The amount of dissolved astaxanthin from the extraction of *H. pluvialis* increased with pressure, i.e. 0.014×10^{-7}

(at 20 MPa) to 18.42×10^{-7} (at 40 MPa) mole of dissolved astaxanthin per mole of CO₂ because an increase in pressure enhanced the density of CO₂ as stated earlier.

Let's now define the extraction efficiency (%) as the ratio between actual dissolved astaxanthin from the extraction of *H. pluvialis* and the solubility of astaxanthin (from the standard) multiplied by 100 at the same pressure and temperature. The extraction will be limited by the level of solubility when this extraction efficiency moves closer to 100%. On the other hand, if this extraction efficiency becomes low, the extraction will not be solubility limited but will be regulated by some other mass transfer mechanism in the process, e.g. high mass transfer resistance between phases. To evaluate for the extraction efficiency of the extraction process provided by Machmudah et al. (2006), let's further focus on the extraction at 70°C over the pressure range of 20 to 40 MPa. The extraction efficiency at 20 MPa was calculated to be around 0.41% whereas this was 29.4% and 21.8% at 30 and 40 MPa, respectively. This observation suggests that the solubility did not affect the extraction process at low pressure (20 MPa), rather, the solubility became more significant in controlling the extraction at higher pressure. Increasing the pressure from 30 to 40 MPa did not see much deviation in the extraction efficiency which could infer that solubility might not be the only one controlling mechanism in this process, and the other limiting factors such as mass transfer resistance also had a consistent share in manipulating the extraction process.

4.2 Co-solvent:

Due to slightly polar solute of astaxanthin, a few research aimed to increase solvent power of astaxanthin in SC-CO₂ extraction by addition of other co-solvent such as ethanol (Machmudah et al., 2006) and vegetable oil (Krichnavaruk et al., 2008). The defined extraction efficiency (%) as stated earlier in Section 4.1 can be applied to explain the extraction efficiency of astaxanthin extraction using co-solvents. Table 2 shows the evaluation of astaxanthin extraction efficiency by ethanol as co-solvent. Increasing in ethanol content in the solvent mixture could enhance the solubility of astaxanthin and exhibited the maximum percent extraction efficiency at around 43.53 % at 5 % ethanol. A further increasing ethanol content in the solvent mixture did not seem to give positive influence to extraction efficiency. It is due to an increasing solubility of astaxanthin in the solvent mixture of ethanol and SC-CO₂, however; a very high amount of ethanol content in the solvent mixture lowers the density of the supercritical fluid, and in some cases, the two phases occurs instead of one phase of solvent mixture results

to lower extraction efficiency as illustrated in Table 2 [22].

Another case is to utilize soybean oil as co-solvent in extraction process. the results of %extraction efficiency are given in Table 3. %Extraction efficiency increased with amount of soybean oil content in the solvent mixture and obtained the maximum around 33.73% at 12% soybean oil content. This is because of two reasons; one is that the solubility of astaxanthin increased in the mixture of the solvent mixture and the other one is the swollen alga matrix effect caused by the solvent mixtures. This finding agreed with Bamberger et al. [23]'s research who claimed that the solubility of a less volatile lipid component was significantly enhanced by the presence of a more volatile triglyceride species in the system. Furthermore, soybean was a good solvent for astaxanthin in the liquid state, considering an appropriate co-solvent for SC-CO₂ extraction [7].

Table 1 Solubility data correlation from solubility model by EOS

Temperature (°C)	P (MPa)	c_{12}	k_{12}	Solubility, $y_2 \times 10^7$ (mole of astaxanthin/mole of CO ₂)	%AARD
80	40	-0.656	-0.09	89.96	0.00
	30	-0.656	-0.06	39.76	
	20	-0.656	-0.05	3.50	
60	40	-0.656	-0.11	66.55	0.03
	30	-0.656	-0.07	25.24	
	20	-0.656	-0.03	2.98	
40	40	-0.656	-0.16	32.46	0.07
	30	-0.656	-0.12	14.51	
	20	-0.656	-0.09	1.65	

Table 2 Extraction efficiency of astaxanthin extraction from *H. pluvialis* at 70°C 40 MPa by using different percentages of ethanol as SC-CO₂:co-solvent [6, 7]

Ethanol (% v/v)	% Extraction Efficiency
0	21.80
3.3	13.60
5.0	43.53
7.5	38.09
10.0	35.35

Table 3 Extraction efficiency of astaxanthin extraction at 70°C 40 MPa from *H. pluvialis* by using different percentages of soybean oil as SC-CO₂:co-solvent (Krichnavaruk et al., 2008)

Soybean oil (% v/v)	% Extraction Efficiency
0	21.76
6	15.23
10	32.64
12	33.73

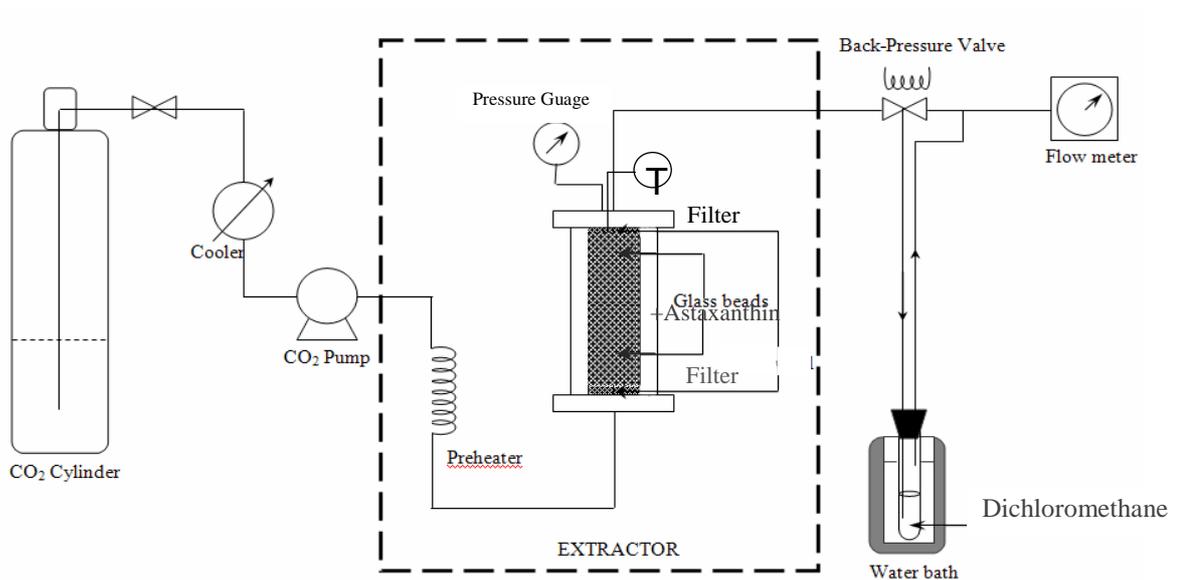


Figure 1 Experimental setup of astaxanthin solubility in pure liquid CO₂ measurement by dynamic technique

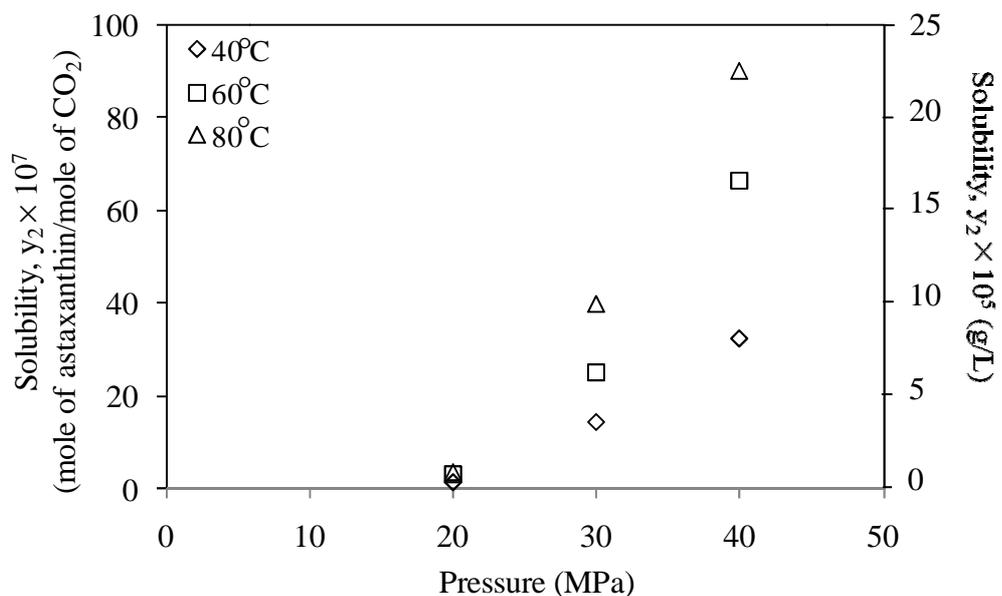


Figure 2 Effect of pressure and temperature on solubility of astaxanthin

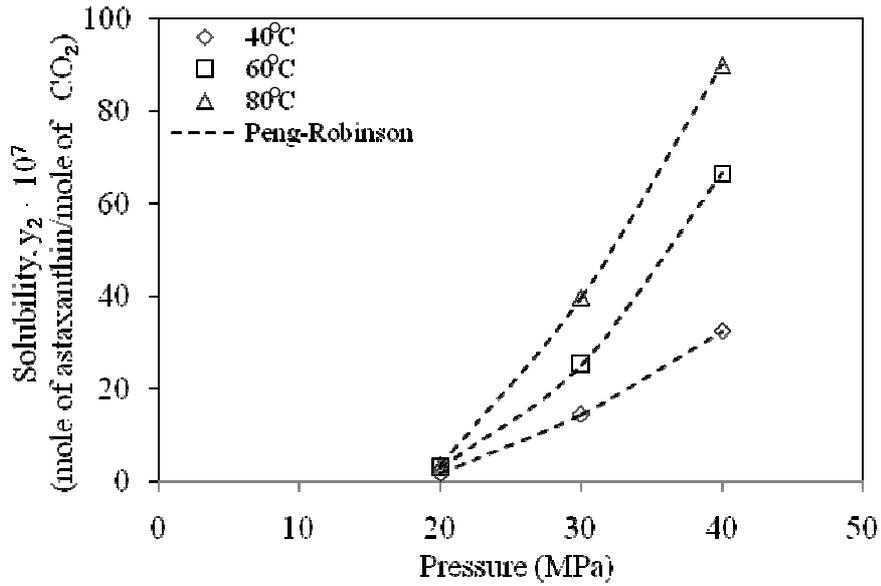


Figure 3 Correlation between solubility of astaxanthin and pressure using EOS model

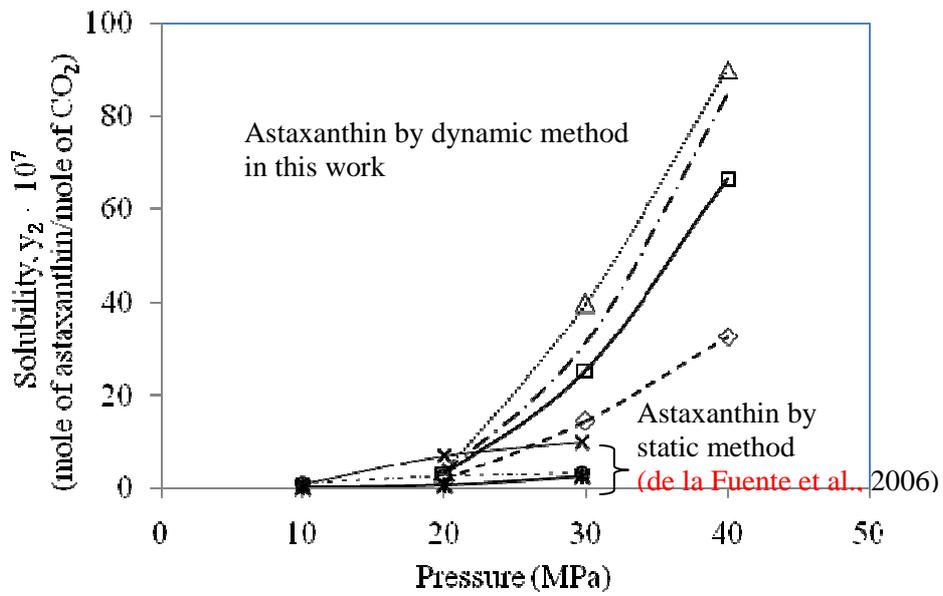


Figure 4 Comparison between solubility of astaxanthin from this work and de la Fuente et al.'s research [8]

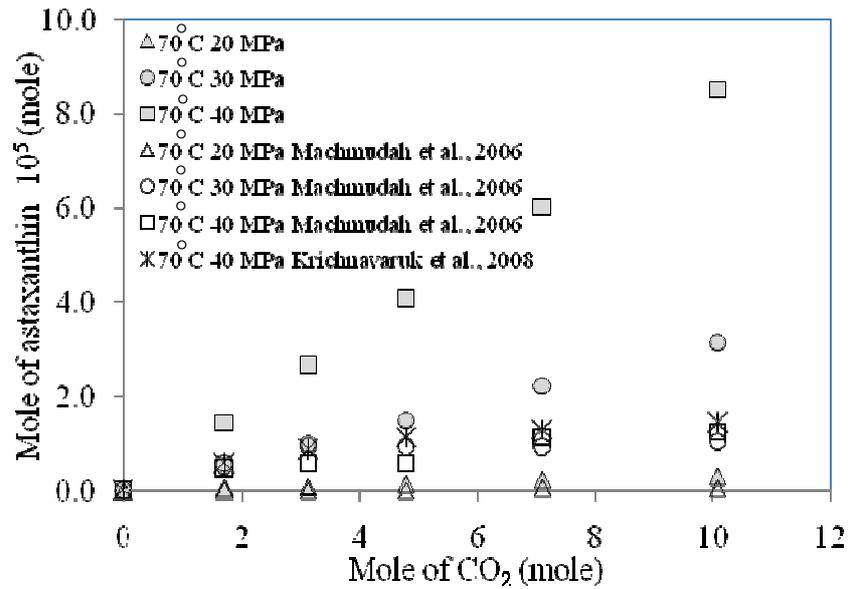


Figure 5 Overall extraction curve of astaxanthin from *H. pluvialis* [6, 7] and standard astaxanthin (this work) at 70°C

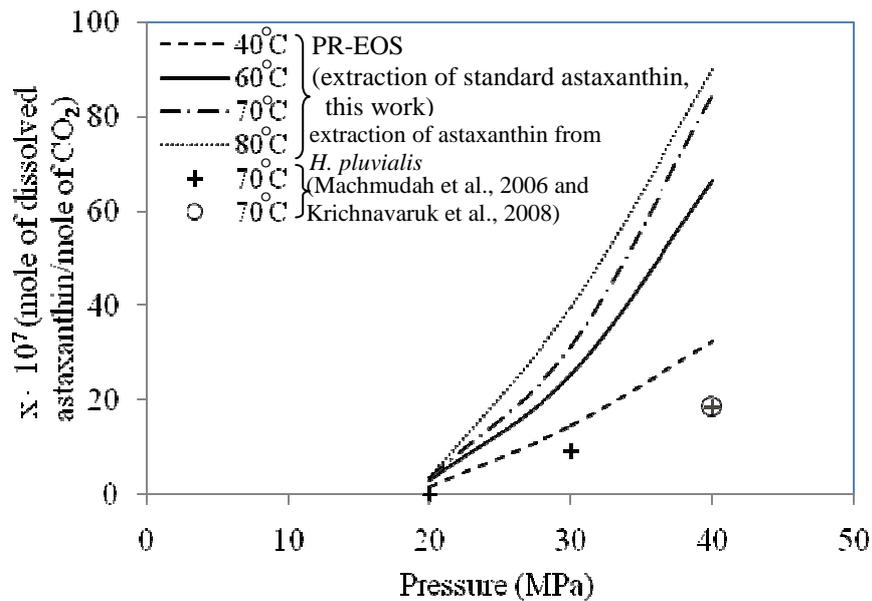


Figure 6 Comparison between dissolved astaxanthin standard and dissolved astaxanthin from *H. pluvialis* in carbon dioxide [6, 7]

CONCLUSION

The model based on Peng-Robinson equation of state was found to best describe the solubility of astaxanthin over the entire range of temperature and pressure examined. Such optimal solubility model was employed to evaluate the extraction efficiency of the reported experimental data. In supercritical CO₂ extraction process, the solubility of astaxanthin from *H. pluvialis* was enhanced by mixing of co-solvent such as ethanol and soybean oil; however, the co-solvent and SC-CO₂ ratio were also important as it significantly affected %extraction efficiency. Moreover, in supercritical CO₂ extraction process, the solubility of astaxanthin *H. pluvialis* was limited due to the mass transfer limitation which rendered the extraction a highly time consuming process.

REFERENCES

- [1] R.T. Lorenz, G.R. Cysewski, Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin, *TIBTECH* 18 (2000) 160-167.
- [2] N. Shimidzu, M. Goto, W. Miki, Carotenoids as singlet oxygen quenchers in marine organisms, *Fisheries Science* 62 (1996) 134-137.
- [3] H. Jyonouchi, S. Sun, K. Iijima, D.M. Gross, Antitumor activity of astaxanthin and its mode of action, *Nutrition and Cancer* 36(1) (2000) 59–65.
- [4] B. Nobre, F. Marcelo, R. Passos, L. Beirão, A. Palavra, L. Gouveia, R. Mendes, Supercritical carbon dioxide extraction of astaxanthin and other carotenoids from the microalga *Haematococcus pluvialis*, *European Food Research and Technology* 223 (2006) 787–790.
- [5] M.A. McHugh, V.J. Krukonis, *Supercritical Fluid Extraction: Principles and Practice*, Butterworth–Heinemann, Massachusetts, 1994.
- [6] S. Machmudah, A. Shotipruk, M. Goto, M. Sasaki, T. Hirose, Extraction of astaxanthin from *Haematococcus pluvialis* using supercritical CO₂ and ethanol as entrainer, *Industrial & Engineering Chemistry Research* 45 (2006) 3652-3657.
- [7] S. Krichnavaruk, A. Shotipruk, M. Goto, P. Pavasant, Supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis* with vegetable oils as co-solvent, *Bioresource Technology* 99 (2008) 5556–5560.
- [8] J.C. de la Fuente, B. Oyarzún, N. Quezada, J.M. del Valle, Solubility of carotenoid pigments (lycopene and astaxanthin) in supercritical carbon dioxide, *Fluid Phase Equilibria* 247 (2006) 90–95.

- [9] H.Youn, M. Roh, A.Weber, G.T. Wilkinson, B. Chun, Solubility of astaxanthin in supercritical carbon dioxide, *Korean Journal of Chemical Engineering* 24(5) (2007) 831-834.
- [10] K.D. Bartle, A.A. Clifford, S.A. Jafar, G.F.Shistone, Solubilities of solids and liquids of low volatility in supercritical carbon dioxide, *Journal of Physical and Chemical Reference Data* 20 (1991) 728-756.
- [11] J. Chrastil, Solubility of solids and liquids in supercritical gases, *Journal of Physical Chemistry* 86 (1982) 3016-3021.
- [12] J. M. Prausnitz, R.N.Lichtenthaler, E.G. Azevedo, *Molecular Thermodynamics of Fluid-Phase Equilibria*, Prentice-Hall, New Jersey, (1986).
- [13] H. Sovová, R. P. Stateva, A.A. Galushko., Solubility of β -carotene in supercritical CO₂ and the effect of entrainers, *Journal of supercritical fluids* 21(2001) 195-203.
- [14] R. Murga, M.T.Sanz, S. Beltrán, J.L. Cabezas., Solubility of some phenolic compounds contained in grape seeds in supercritical carbon dioxide, *Journal of Supercritical Fluids* 23 (2002) 113-121.
- [15] R.B. Gupta and J. Shim, *Solubility in Supercritical Carbon Dioxide*, CRC press Taylor&Francis Group, New York, 2007.
- [16] J. Shi, C. Yi, S.J. Xue, Y. Jiang, Y. Ma, Dong Li, Effects of modifiers on the profile of lycopene extracted from tomato skins by supercritical CO₂, *Journal of food engineering*, 93 (2009) 431-436.
- [17] Ö. Güçlü-Üstündağ, and F. Temelli, Correlating the solubility behavior of minor lipid components in supercritical carbon dioxide, *Journal of Supercritical Fluids* 31(2004) 235–253.
- [18] L. Danielski, L.M.A.S. Campos, L.F.V. Bresciani, H. Hense, R.A. Yunes, S.R.S. Ferreira, Marigold (*Calendula officinalis* L.) oleoresin: Solubility in SC-CO₂ and composite profile. *Chemical Engineering and Processing* 46 (2007) 99-106.
- [19] R.C. Reid, J.M. Prausnitz, B.E. Poling, *The properties of Gases and Liquids*, McGraw-Hill, New York, 1987.
- [20] B.E. Poling, J.M. Prausnitz, J.P.O'connell, *The Properties of Gases and Liquids*, McGrawHill, London, 2001.
- [21] D. Ruen-ngam, Comparison of Extraction Methods for Recovery of Astaxanthin from *Haematococcus pluvialis*, PhD Thesis, Chulalongkorn University, Bangkok, Thailand, November 8, 2010.

- [22] G. Brunner, *Gas Extraction*; Springer, Steinkopff: Darmstadt, Germany, New York, **1994**.
- [23] T. Bamberger, C.J. Erickson, L.C. Cooney, Measurement and model prediction of solubilities of pure fatty acids, pure triglycerides, and mixtures of triglycerides in supercritical carbon dioxide. *J. Chem. Eng. Data* 33 (**1988**) 327–333.