Korean J. Chem. Eng., **24**(5), 831-834 (2007)
JOURNAL REVIEW

Solubility of astaxanthin in supercritical carbon dioxide

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(*Received 4 August 2006 • accepted 9 February 2007*)

Abstract–The solubility of astaxanthin in carbon dioxide was measured under supercritical conditions of a pressure range from 80 to 300 bar, and temperature range from 303 to 333 K, by using a dynamic flow-type. The solubility of astaxanthin increasing from 0.42×10^{-5} to 4.89×10^{-5} with higher temperature and pressure maintains certain density of supercritical carbon dioxide. The solubility data obtained were applied to the Chrastil model, based on the density of carbon dioxide. The data fitted well with the Chrastil model at most experimental conditions.

Key words: Astaxanthin, Supercritical Carbon Dioxide, Solubility, Chrastil Model

INTRODUCTION

Carotenoid is a generic name used to designate one of the most common groups of naturally occurring pigments found in animals and plants. Most carotenoids are unsaturated polyhydrocarbon containing 40 carbon atoms and two terminal ring systems. Carotenoids are also highly conjugated polyprenoid nutrients essential to the human diet by virtue of their antioxidant and anti-cancer properties [1].

Carotenoids composed entirely of carbon and hydrogen are known as carotenes, whereas containing oxygen are termed xanthophylls. Astaxanthin (3,3'-dihydroxy-β-β-carotene-4,4'-dione) is a xanthophyll which is widely distributed in nature and is the principal pigment found in crustaceans. It is a strong antioxidant as well as a common keto-carotenoid found in a wide range of algae, invertebrates, fish and crustacea. Astaxanthin can be recovered from crustacean processing wastes. The previous studies suggest that it has higher antioxidant activity by about 10-fold than other carotenoids [2-4].

Among marine carotenoid pigments, astaxanthin is the most common xanthophyll. It contributes to the red or pink color in the carapaces of crustaceans, integuments of teleosts, muscles of salmonoids, and ovaries of fish and shellfish [5-9]. This pigment has been recovered for industrial uses mainly as an agent for the pigmentation of cultured fish and shellfish [10,11].

Recently, further diverse biological functions have been revealed including that of a vitamin A precursor [12], a scavenger and/or quencher of free radicals and active oxygen [13,14], a preventative against cancer [15,16], and an enhancer of the immune response systems [17,18]. The quenching effect of astaxanthin for molecular oxygen is approximately 500 to 1,000 times greater than that of α-tocopherol [19]. This carotenoid has potential applications in both the pharmaceutical and food industries.

Currently, astaxanthin is commonly extracted by liquid solvent extraction using toluene, hexane or petroleum ether. However, the conventional method is paid much attention, from the discharge of potentially hazardous solvents to the environment and damage to the functional properties of the extracts by hydrothermal stress [20, 21]. Therefore, other extraction techniques with better selectivity and efficiency have been sought.

Supercritical fluid extraction (SFE) is an alternative separation technology. The extract obtained from SFE contains fewer polar impurities than the current organic liquid extract, and then subsequent purification steps become easier [22]. SFE is more selective for carotenes than organic liquid extraction and is preferred for handling temperature-sensitive molecules. Supercritical carbon dioxide ($S\text{-}CO_2$), in particular, has further processing advantages such as low viscosity, low surface tension, high diffusivity and good density, which play key roles in enabling the solvent to readily penetrate the solid biomass matrix as well as in extracting the solutes. $S\text{-}CO_2$ with moderate critical temperature and pressure (304.2 K, 73.8 bar) is non-toxic and largely inert in these systems, not flammable and inexpensive [23-30]. The aim of this work was to obtain fundamental data on the solubility of astaxanthin in $S\text{-}CO_2$ under various pressures and temperatures in the range of commercial interest and then to apply the data to the recovery of astaxanthin from natural resources.

EXPERIMENTAL

1. Materials

Astaxanthin with purity above 98% was used without purification. Solvents and reagents were HPLC grade. SFE grade CO_2 (>99.9%) was used as an extraction fluid. The chloroform (>99.9%) was used without further purification.

2. Apparatus and Procedures

The solubility of astaxanthin was measured by using the semi-continuous flow type apparatus shown in Fig. 1. Samples containing 0.2 g astaxanthin dissolved in 3 ml of chloroform were absorbed onto 1.0 g of cotton wool and loaded into the 10 ml extractor. The pressurized CO_2 was preheated in the constant temperature chamber prior to sample loading. The pressure in the extractor was controlled by a needle valve just prior to the separator and the flow rate

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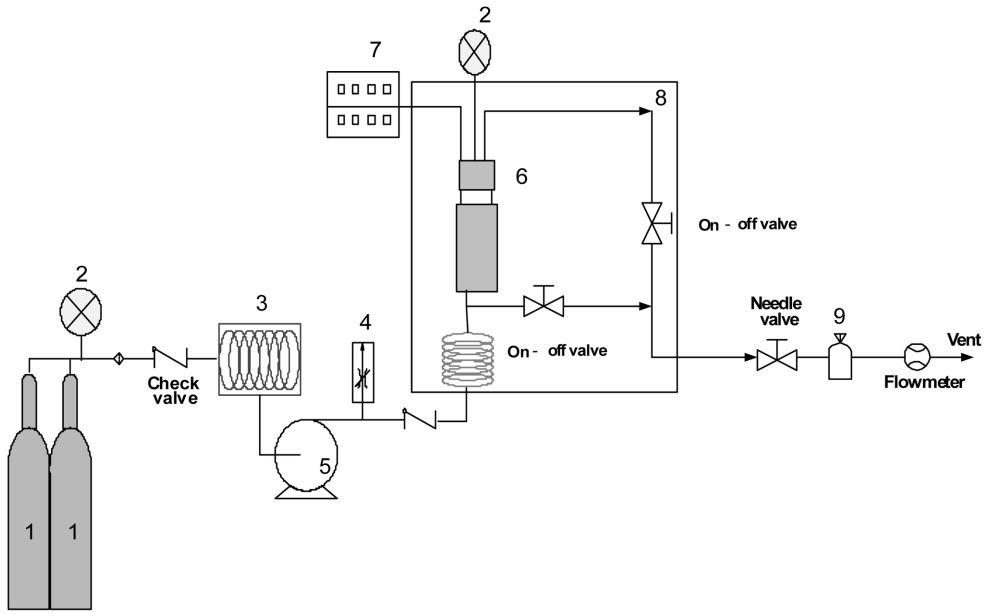

Fig. 1. Solubility measurement apparatus process flow diagram.

1. CO_2 tank	3. Cooling unit	5. Pump	7. Thermometer	9. Separator
2. Pressure gauge	4. Safety valve	6. Reactor	8. Thermostat	

by the metering pump. Pressure ranged 80-300 bar ±0.1% fsd. The extractor temperature in the range 303-333 K was measured by a K-type thermocouple. The extracts which accumulated in the separator were weighed at 5 minute intervals. The total flow rate of CO_2 was measured by a dry gas meter.

RESULTS AND DISCUSSION

Table 1 shows the solubility data of astaxanthin in terms of temperature and pressure. Mole fractions of astaxanthin in carbon dioxide were calculated by using the following equation:

$$y_{astaxanthin} = \frac{n_{astaxanthin}}{n_{astaxanthin} + n_{CO_2}} \quad (1)$$

where: $y_{astaxanthin}$ represents the mole fraction of astaxanthin, and $n_{astaxanthin}$ and n_{CO_2} are moles of astaxanthin and carbon dioxide, respectively. Interestingly, the solubility of astaxanthin lies in the range of 10^{-6} to 10^{-5} which is similar to that of β-carotene [31]. Astaxanthin and β-carotene are similar biological compounds. The values of solubility obtained here on mixtures were more than those reported by Saldaña et al. [32] which were obtained by using a binary and a multi component complex system.

The detailed plots of astaxanthin solubility vs. density shown in Fig. 2 clearly show the isotherms and the effects of temperature and solvent density. At constant temperature, solubility increases more than proportionally with density and the proportional increase in solubility with temperature is greater at higher densities. Thus, a solubility increase is better obtained by an increase in density of the solvent rather than a temperature increase, with the added advantage of limiting thermal degradation.

The Chrastil equation [33] was applied to the astaxanthin solubility data. Despite its limitations the equation has proved useful in SFE work and is easy to use. The density relationship of Chrastil is based on the following equation (derived from Antoine):

$$y_{astaxanthin} = \rho_{CO_2}^{k} \exp\left(\frac{a}{T} + b\right) \quad (2)$$

where $y_{astaxanthin}$ is the astaxanthin solubility (mol/mol), ρ_{CO_2} the supercritical carbon dioxide density, T is experimental temperature (K) and a, b and k empirical fitting parameters. The solubility data fit the Chrastil model quite well. Table 2 gives the Chrastil parameters obtained for astaxanthin.

As another method for modeling the supercritical carbon dioxide extraction, a simple approach was adopted. The enhancement factor, η, was correlated with the corresponding density of the pure CO_2 according to the following equation:

$$\ln \eta = \alpha + \beta \rho_{CO_2} \quad (3)$$

$$\eta = \frac{p y_{astaxanthin}}{p^{o}} \quad (4)$$

The saturated vapor pressure of the solute is represented as p^o, assuming 1 Pa.

The correlation shown in Fig. 3 demonstrates a good linear relationship between ln η and the density of CO_2 across all conditions. The density of CO_2 was obtained from the published data. The coefficients in Eq. (3) are summarized in Table 3. The values of a and b are independent of temperature and are similar to those reported by Iwai et al. [34].

The average absolute relative deviation (AARD) for each data series was calculated as follows:

$$AARD = (100/N) \sum \frac{|y_{i,exp} - y_{i,calc}|}{y_{i,exp}} \quad (5)$$

In the case of astaxanthin the average absolute relative deviations of calculated and experimental data appear in Table 1.

Table 1. Solubility of astaxanthin, as mole fraction ($y_{astaxanthin}$), in CO_2 at temperatures in the range from 303 to 333 K and pressures from 80 to 300 bar

T (K)	P (bar)	ρ_{CO_2} (kg/m^3)	$y_{astaxanthin} \times 10^5$ (mole fraction)	AARD (%)
303	80	702.06	0.78	13
	100	772.10	1.09	
	120	809.52	1.02	
	150	847.55	1.27	
	200	891.08	1.78	
	250	922.96	1.81	
	300	948.53	1.91	
308	80	424.32	0.76	6
	100	714.16	1.43	
	120	768.33	1.46	
	150	816.12	1.65	
	200	866.66	1.91	
	250	902.12	2.09	
	300	929.95	2.10	
313	80	278.41	0.42	7
	100	630.18	1.49	
	120	719.70	1.80	
	150	781.79	1.92	
	200	841.09	2.46	
	250	880.67	2.62	
	300	911.01	2.81	
318	80	278.41	0.48	10
	100	500.19	1.62	
	120	660.39	2.59	
	150	743.99	2.85	
	200	814.27	3.70	
	250	858.59	4.18	
	300	891.71	4.32	
323	80	218.92	0.47	11
	100	384.65	0.80	
	120	587.58	2.53	
	150	702.21	3.06	
	200	786.15	4.01	
	250	835.88	4.53	
	300	982.04	4.62	
328	80	203.02	0.53	6
	100	325.82	0.79	
	120	509.04	1.87	
	150	657.71	2.98	
	200	757.59	3.89	
	250	813.25	4.46	
	300	852.63	4.69	
333	80	191.71	0.80	3
	100	290.09	1.20	
	120	437.10	2.09	
	150	608.34	3.17	
	200	726.88	3.96	
	250	789.27	4.51	
	300	832.30	4.89	

*Density of pure CO_2 obtained from published data [35].

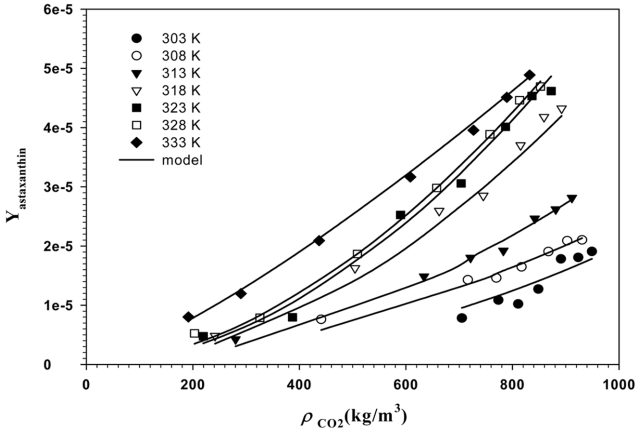

Fig. 2. Astaxanthin solubility in carbon dioxide at different conditions.

Table 2. Parameters in chrastil equation of astaxanthin mole fraction (Eq. (2))

T (K)	k	a	b
303	2.10	1.00	−1.74
308	1.74	1.00	−1.61
313	1.87	1.00	−1.62
318	1.90	1.00	−1.58
323	1.90	1.00	−1.56
328	2.11	0.91	−1.62
333	1.28	0.96	−1.37

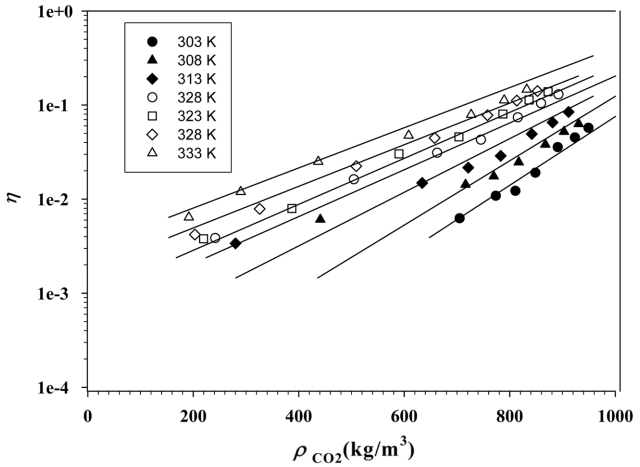

Fig. 3. Relationship between the enhancement factor and density of CO_2 at various temperatures.

CONCLUSIONS

The measured solubility of astaxanthin in supercritical carbon dioxide increased by a factor of twelve from 303 to 333 K and 80 to 300 bar. The solubility of astaxanthin is strongly dependent on solvent density. The solubility data were modeled by using the Chrastil equation with AARD values of 13%, 6%, 7%, 10%, 11%, 6% and 3% for 303 K, 308 K, 313 K, 318 K, 323 K, 328 K, 333 K.

Table 3. Coefficients of Eq. (4) obtained from Fig. 3

Conditions	α (−)	$\beta \times 10^3$ (m^3/kg)
303 K	−11.8	9.4
308 K	−7.4	4.7
313 K	−7.2	5.0
318 K	−6.9	5.3
323 K	−6.9	5.6
328 K	−6.6	5.4
333 K	−5.8	4.7

A simple unit operation, supercritical fluid extraction can replace or be a retrofit to conventional processes, leading to enhanced recovery and increased product quality. However, for successful process design it is important to understand the physical properties of supercritical fluids and the target materials. Based upon the data obtained here a mass transfer model for extracting astaxanthin from crab shell can now be developed and the optimum extraction conditions investigated systematically.

ACKNOWLEDGMENTS

This research was financially supported by a grant (M2007-05) from Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Maritime Affairs & Fisheries, Republic of Korea and the Ministry of Commerce, Industry and Energy (MOCIE) and Korea Industrial Technology Foundation (KOTEF) through the Human Resource Training Project for Regional Innovation.

NOMENCLATURE

$n_{astaxanthin}$: moles of astaxanthin
n_{CO_2} : moles carbon dioxide
$y_{astaxanthin}$: mole fraction of astaxanthin
ρ : density of CO_2 [kg/m^3]
P : pressure [bar]
P^o : saturated vapor pressure of solute
T : temperature [K]
a, b, k : Chrastil constants [Eq. (2)]
η : enhancement factor
$\alpha\beta$: constants [Eq. (3)]
N : number of data points
AARD : average absolute relative deviation [Eq. (3)]
$y_{i,exp}$: mole fraction of i, experimental
$y_{i,calc}$: mole fraction of i, calculated

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