Solubilities of 7,8-Dihydroxyflavone and 3,3′,4′,5,7-Pentahydroxyflavone in Supercritical Carbon Dioxide

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The solubilities of 7,8-dihydroxyflavone and 3,3′,4′,5,7-pentahydroxyflavone (quercetin) in supercritical carbon dioxide (SC-CO_2) were measured at 308.2 K and 318.2 K over the pressure range from 9.1 MPa to 25.3 MPa by a flow type apparatus. The solubilities were determined from the mass of solute trapped by decompression and the volume of CO_2. Solubility data were correlated by a regular solution model.

Introduction

Recently, clinical and biological studies have indicated that flavonoids, such as 7,8-dihydroxyflavone and 3,3′,4′,5,7-pentahydroxyflavone (quercetin), may play a significant role in the prevention of oxidant activity and in many forms of cancer.[1] It is desired to develop the effective method for extraction of flavonoids from natural products. Supercritical fluid extraction (SFE) has attracted much attention as a powerful method for separating mixtures of biomolecules and bioactive constituents from natural products, because it provides biologically mild conditions and efficient recovery of the target substance. Recently, investigators have examined the extraction of natural products.[2] In our previous work,[3] the feasibility of extraction of flavonoids from natural products was demonstrated using supercritical carbon dioxide (SC-CO_2). To aid design and optimization of extraction of flavonoids from natural products, solubilities of flavonoids are needed as a fundamental knowledge. Although a large number of studies have been performed on solubilities of many compounds in SC-CO_2,[4,5] little is known about flavonoids.[6]

In this work, the solubilities of 7,8-dihydroxyflavone and quercetin in SC-CO_2 at 308.2 K and 318.2 K over the pressure range from 9.1 MPa to 25.3 MPa were measured by a flow type apparatus. The solubilities of these substances were correlated by a solution model based on the regular solution model.[7,8]

Experimental Section

Apparatus and Procedures. A flow type apparatus was used to measure the solubilities of 7,8-dihydroxyflavone and 3,3′,4′,5,7-pentahydroxyflavone (quercetin) in supercritical carbon dioxide (SC-CO_2). A detailed description of the apparatus and operating procedures are given in our previous paper.[6,9−11] The liquefied carbon dioxide (CO_2) leaving a gas cylinder was passed through a cooling unit to prevent vaporization of CO_2 from warming up, and it was directed to a compressor which was capable of delivery pressures up to about 60 MPa and delivery rates up to 5.2 mL·min^{-1} (liquefied CO_2 basis). A back-pressure regulator was used to maintain a constant pressure with the pressure control accuracy of ±0.1 MPa. The equilibrium pressure was measured by a Bourdon gauge calibrated against a strain pressure gauge (accuracy ±0.3%). CO_2 passed through a preheating coil and then entered in two equilibrium cells containing 7,8-dihydroxyflavone or quercetin. The cells were constructed from 316 Stainless Steel, and the inner diameter, height, and volume were 30 mm, 30 cm, and 210 cm^3, respectively. The solid component was packed in the second cell with glass beads to prevent channeling, while the first cell was used as a buffer tank. These cells were attached to a preheating coil and submerged in a water bath controlled with a temperature control accuracy of ±0.1 K. The supercritical fluid (SCF) saturated with the solid component was decompressed through an expansion valve and introduced into a U-shaped glass tube cooled in an ice bath. Gaseous CO_2 and the solid component were separated in the tube. The amount of the trapped solid component was determined by mass. The solute collected in the tubes was weighed by a direct reading balance (Exact A-V; accuracy 0.1 mg). The volume of CO_2 was measured by a wet-gas meter that was calibrated to an accuracy of ±0.4%. Usually 0.1 to 0.3 g of solute was trapped, and the flow rate of expanded CO_2 was adjusted to be 0.30−0.85 L·min^{-1} (gaseous CO_2 basis). A small amount of the solid remaining in the tube and the expansion valve was removed and trapped by using SC-CO_2 through a bypass line.

Materials. 7,8-Dihydroxyflavone and 3,3′,4′,5,7-pentahydroxyflavone (quercetin) were purchased from Tokyo Kasei Kogyo Co. Ltd. Their purities were believed to be more than 98%. High-purity CO_2 (more than 99%, Fukuoka Sanso Co. Ltd.) was used as received.

Results and Discussion

The measurements were carried out for several flow rates of CO_2 at known pressure. The solubilities of 7,8-

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Table 1. Mole Fraction Solubilities, y_2, of 7,8-Dihydroxyflavone and 3,3′,4′,5,7-Pentahydroxyflavone in SC-CO_2

7,8-dihydroxyflavone				3,3′,4′,5,7-pentahydroxyflavone			
T = 308.2 K		T = 318.2 K		T = 308.2 K		T = 318.2 K	
p/MPa	10^5y_2	p/MPa	10^5y_2	p/MPa	10^5y_2	p/MPa	10^5y_2
9.1	0.862	10.1	1.60	10.1	0.610	10.1	0.542
10.1	1.21	15.2	3.21	15.2	0.965	15.2	1.88
15.2	1.51	20.3	3.60	20.3	1.05	20.3	2.14
20.3	1.80	25.3	3.88	25.3	1.20	25.3	2.20
25.3	1.87						

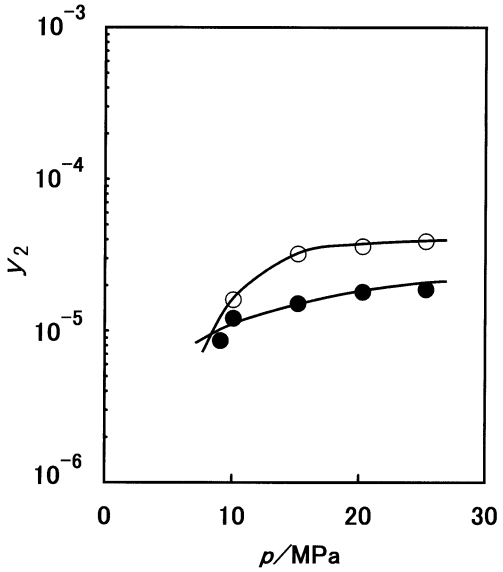

Figure 1. Mole fraction solubilities of 7,8-dihydroxyflavone in SC-CO_2: ●, experimental data at 308.2 K; ○, experimental data at 318.2 K; —, calculated results.

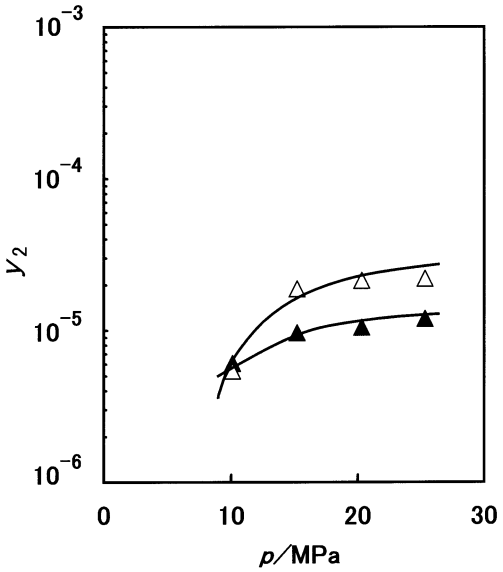

Figure 2. Mole fraction solubilities of 3,3′,4′,5,7-pentahydroxyflavone in SC-CO_2: ▲, experimental data at 308.2 K; △, experimental data at 318.2 K; —, calculated results.

dihydroxyflavone and 3,3′,4′,5,7-pentahydroxyflavone were independent of the CO_2 flow rate. Similar results were obtained for flavone and 3-hydroxyflavone.[6] The experimental values of solubilities of 7,8-dihydroxyflavone and quercetin listed in Table 1 were obtained from an arithmetic average of several measurements at each pressure. The reproducibility of these solubilities was within ±4.0%

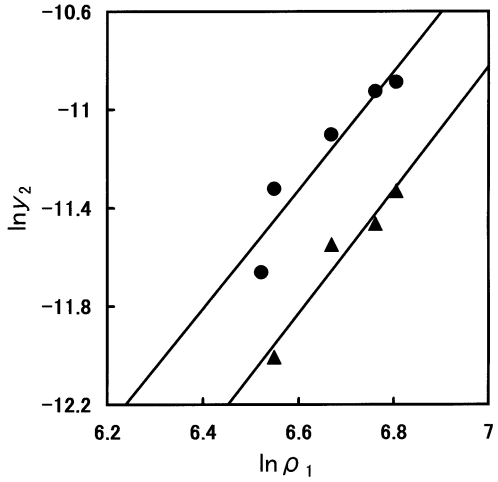

Figure 3. Relationship between solubilities, y_2, and density of pure CO_2, ρ_1: ●, 7,8-dihydroxyflavone at 308.2 K; ▲, 3,3′,4′,5,7-pentahydroxyflavone at 308.2 K; —, results calculated by eq 1.

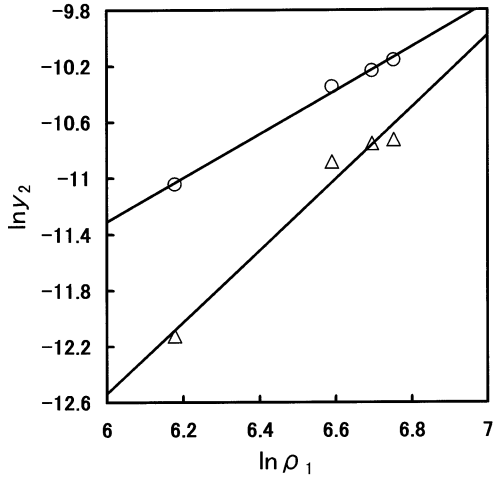

Figure 4. Relationship between the solubilities, y_2, and density of pure CO_2, ρ_1: ○, 7,8-dihydroxyflavone at 318.2 K; △, 3,3′,4′,5,7-pentahydroxyflavone at 318.2 K; —, results calculated by eq 1.

when the pressure was varied from 9.1 MPa to 25.3 MPa. Experimental data of 7,8-dihydroxyflavone and quercetin at 308.2 K and 318.2 K are given in Figures 1 and 2. Just above the critical pressure of CO_2, the solubilities of 7,8-dihydroxyflavone and quercetin drastically increase because of a rapid increase in density with pressure. For pressures above 10 MPa, the solubility increases with temperature because the decrease in CO_2 density cannot overcome the increase in vapor pressure. Compared with our previous solubility data of flavone and 3-hydroxyflavone,[6] those of 7,8-dihydroxyflavone and quercetin are decreased with an increase of the hydroxyl group in the flavonoids.

To confirm the reliability of the experimental data, the solubility of the solid component in the SCF, y_2, was plotted against the density of pure CO_2, ρ_1. Empirical equations for the solubility have been proposed as a function of density for a SCF.[12] The density of CO_2 was calculated by the equation of state of Angus et al.[13] As shown in Figures 3 and 4, it was observed that the logarithm of the solubility, ln y_2, was a linear function of the logarithm of the solvent density, ln ρ_1. The relationship can be represented by the following equation:

$$\ln y_2 = a \ln \rho_1 + b \qquad (1)$$

Table 2. Coefficients in Eq 1

substance	T/K	a	b
7,8-dihydroxyflavone	308.2	2.413	−27.25
	318.2	1.561	−20.68
3,3′,4′,5,7-pentahydroxyflavone	308.2	2.514	−28.42
	318.2	2.558	−27.89

Table 3. Physical Properties of 7,8-Dihydroxyflavone and 3,3′,4′,5,7-Pentahydroxyflavone

substance	T_2^{m} [a] / K	H_2^{m} [b] / J·mol^{-1}	ΔU^* [c] / J·mol^{-1}
7,8-dihydroxyflavone	558.1	34 500	33 360
3,3′,4′,5,7-pentahydroxyflavone	587.1	40 900	49 020

[a] Nihon-Kagakukai.[16] [b] Extrapolated values by using ΔH_2^m from coal liquefaction products. [c] Calculated by the group contribution proposed by Fedors[15] at 298.15 K.

where the coefficients a and b are given in Table 2. However, good calculation results were not obtained for 7,8-dihydroxyflavone in the low pressure region, as shown in Figure 3. It may be considered that the solubility of flavonoids is strongly dependent on the operating pressure near the critical pressure of CO_2, and good experimental data were not obtained.

Correlation

The regular solution model coupled with the Flory−Huggins theory[7,8] is applied to correlate the solubilities in SC-CO_2. The solubility, y_2, in SC-CO_2 at infinite dilution conditions can be calculated by the following equation.

$$\ln y_2 = \frac{\Delta H_2^{m}}{RT}\left(\frac{T}{T_2^{m}} - 1\right) - \frac{v_2}{RT}(\delta_1 - \delta_2)^2 - 1 + \frac{v_2}{v_1} - \ln\frac{v_2}{v_1} \quad (2)$$

where T is the absolute temperature, R is the universal gas constant, T_2^{m} is the melting point of the solid, ΔH_2^{m} is the enthalpy of fusion, δ_1 is the solubility parameter of SC-CO_2 calculated by the method proposed by Giddings et al.,[14] and v_1 is the molar volume of SC-CO_2 calculated with the equation of state proposed by Angus et al.[13] To simplify the calculation by the solution model proposed by Iwai et al.,[7] the solubility of solute in SC-CO_2 was assumed as the dilution condition. This assumption may be reasonable, because the solubility of solid compounds in SC-CO_2 is very little (less than 10^{-3}). δ_2 is the solubility parameter of the solid component calculated by

$$\delta_2 = \sqrt{\frac{\Delta U_2}{v_2}} \quad (3)$$

where the value of ΔU_2 can be calculated by the following equation.

$$\Delta U_2 = \Delta U_2^*\{1 + 1.13\alpha^{v}(T^* - T)\}^2\{1 - \alpha^{v}(T^* - T)\} \quad (4)$$

ΔU_2^* is the internal energy change of vaporization at $T^* = 298.15$ K, and α^v is the isobaric thermal expansion coefficient. The value of ΔU_2^* can be calculated by Fedors' method.[15] T_2^{m}, ΔH_2^{m}, and ΔU_2^* of 7,8-dihydroxyflavone and quercetin are given in Table 3. The value of α^v was assumed to be 1.0×10^{-3} K^{-1} for simplification. The molar volume of solid, v_2, is treated as an adjustable parameter for optimizing the solubility of the solid component. According to Iwai et al.,[7] the relationship between the molar volume, v_2, and the density of SC-CO_2, ρ_1, can be approximated by the following equation.

$$\ln(v_2/\mathrm{m^3\cdot mol^{-1}}) = \alpha \ln(\rho_1/\mathrm{kg\cdot m^{-3}}) + \beta \quad (5)$$

Table 4. Optimized Values of α and β in Eq 5

substance	T/K	α	β	100σ[a]
7,8-dihydroxyflavone	308.2	−3.696	6.349	8.13
	318.2	−2.630	−0.6789	2.05
3,3′,4′,5,7-pentahydroxyflavone	308.2	−3.547	−1.940	24.8
	318.2	−1.396	−0.2060	7.80

[a] $\sigma = (1/N)\Sigma^N|y_{exp} - y_{calc}|/y_{exp}$, where N is the number of data points.

The values of α and β are listed in Table 4. The solubilities of 7,8-dihydroxyflavone and quercetin in SC-CO_2 can also be well represented by the regular solution model, as shown in Figures 1 and 2.

Conclusion

The solubilities of 7,8-dihydroxyflavone and quercetin in SC-CO_2 were measured by using a flow type apparatus at 308.2 K and 318.2 K over the pressure range from 9.1 to 25.3 MPa. Furthermore, the solubilities of these substances in SC-CO_2 have been correlated by using a regular solution model. It was shown that the solubilities of these substances could be correlated with fairly good accuracy.

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