Scientific paper

Solubility of Carnosic acid and Carnosol from Rosemary Extract in Supercritical CO_2

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Abstract

Rosemary leaves extract are known to have significant antioxidant properties and are widely used in foods and nutritional supplements. Compounds responsible for these antioxidant properties are mainly the phenolic diterpenes. The major phenolic diterpenes are carnosic acid and carnosol. Much interest has been focused on the isolation of carnosic acid, due to its wide spectrum of actions and beneficial effects on human health.

In this work a natural rosemary extract, containing 73.9% carnosic acid and 14.7% carnosol, was investigated regarding the solubility of carnosic acid and carnosol in supercritical CO_2. The solubility measurements, using a static-analytic method, were performed at temperatures 40, 60 and 80 °C and at pressures ranging from 98 to 402 bar. The Chrastil model was used to correlate the obtained experimental solubility data.

Keywords*:* Solubility, carbon dioxide, carnosic acid, carnosol, rosemary extract

1. Introduction

The oxidative deterioration of lipids is a main factor, which determines the loss of product quality. Lipid oxidation is responsible for generation of free radicals and can result in alterations of organoleptic characteristics. In addition, oxidised lipids may have undesiderable effects on the human health.[1,2] Search for natural additives, especially of plant origin, has notably increased in recent years. Rosemary leaves extracts, *Rosmarinus officinalis* L., are known to have significant antioxidant properties due to the content of phenolic diterpenes and are widely used in foods, nutritional supplements and cosmetics.[3–6] A number of authors have reported the effectiveness of rosemary for obtaining higher sensory scores and lowering lipid oxidation in various foods.[7–14]

Complex phenols include a high variety of natural phenolic compounds among which diterpene phenols form an important subgroup. In rosemary extracts, carnosic acid and carnosol are typical representatives of diterpene phenols; their isolation is of great interest because of a wide spectrum of actions, including antimicrobial, anticancer and antimutagenic effects, as well as inhibitory effects on HIV-1 protease.[15,16] It was observed, that carnosic acid is generally poorly stable. Results of the degradation are, at least to some extent, carnosol and other phenolic diterpenes like rosmanol, epirosmanol and methoxyepirosmanol, which are often present in rosemary extract in small concentration.[17–21]

Supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) are technologies, which are lately becoming highly interesting due to several advantages compared to conventional processes. SFE for selective isolation of antioxidants from rosemary has been extensively researched, mainly because of the mild conditions which avoid oxidation and/or degradation of phenolic compounds.[18,22] The pure carnosic acid can be isolated from rosemary extracts using SFE followed by SFC.[23] Among all applied gases and liquids, CO_2 remains the most commonly used fluid as a solvent for SFE applications, chromatography, extractive applications, high-pressure micronisation, and as a (bio)chemical reaction media. This is due to its low critical parameters, non-toxic and non-flammable properties and its availability in high purity. Furthermore, SFE has important advantages over conventional extraction techniques. Tipsrisukond and co-

workers[24] have reported that supercritical fluid extracts have higher antioxidant power than extracts obtained by classical methods.

The solvent power of supercritical fluid generally rises with increasing density. The selection of process parameters (temperature and pressure) is the key factor, which influences the extraction process and quality of extract.[25] Knowledge of carnosic acid's solubility in the supercritical phase is important for the design of a separation process. Cháfer and coworkers[26] studied the solubility of carnosic acid in supercritical CO_2 with ethanol as a modifier. Using SC CO_2 in the SFE process, they found that the solubility of carnosic acid in CO_2 increases with pressure and the amount of ethanol. Furthermore, the solubilities in the investigated pressure range were higher at lower temperatures. Ramirez and coworkers[27] investigated the isolation of phenolic antioxidant compounds (carnosic acid) by supercritical fluid chromatography. By theoretical analysis, they estimated carnosic acid's solubilities in pure CO_2.

In this work, the solubilities of carnosic acid and carnosol from rosemary extract were measured in supercritical CO_2 at temperatures 40, 60 and 80 °C and in pressure range from 98 to 402 bar. The influence of process parameters on the separation was evaluated.

2. Experimental

2. 1. Materials

Natural rosemary extract in powderous form was obtained from Vitiva (Markovci, Slovenia). The content of two major rosemary antioxidative compounds, carnosic acid and carnosol, was 73.9% and 14.7%, respectively. Analytical standard of carnosic acid and carnosol (No. ASB-00003198-010) was supplied by Vitiva (Markovci, Slovenia).

CO_2 (purity 99.97%) was obtained from Linde plin (Celje, Slovenia). All other chemicals were purchased from Merck.

2. 2. Determination of the Equilibrium Solubilities

A static-analytic method was used for measuring solubilities of carnosic acid and carnosol from rosemary extract in supercritical CO_2. A detailed description of the experimental procedure is presented in literature.[28,29] A 500 mL autoclave designed for pressures up to 450 bar and temperature up to 200 °C was loaded with a sufficient amount of substance. CO_2 from the supply tank was cooled to a liquid state and charged into the autoclave by a high pressure pump. Content of the autoclave was mixed under constant operating conditions (temperature and pressure) until equilibrium was reached. After 1 h of phase separation, a sample was taken by the use of a sampling valve into a trap with a solvent (acetonitrile:water = 70:30 v/v acidified with 1.2 mL H_3PO_4) where the components were solubilised. The amount of carbon dioxide released (approx. 1 L) was measured with a gas meter (accurate to 0.02 L). The pressure drop observed when taking samples was between 3 and 6 bar, depending on the pressure in the autoclave. A temperature change was not detected. Since the quantity of the sample was sufficiently small, compared to the volume of the autoclave, further experiments could have been performed. The concentration of carnosic acid and carnosol in acetonitrile-water solution was determined by HPLC.

2. 3. HPLC analysis

The method was similar to that used by Hadolin Kolar and co-workers[30] and Škrinjar and co-workers[31]. The HPLC system for the detection of carnosic acid and carnosol consisted of a SpectraSERIES P100 (Thermo Separation Products) pump, UV-VIS detector UV 1000 (Thermo Separation Products) and a Rheodyne injector, model 7725i (Thermo Separation Products). A Kromasil 100 C18 (250 × 4.6 mm, 5 μm) column (B. I. A., Slovenia) was used.The mobile phase was a mixture of acetonitrile and water (70:30 v/v) and contained 1.2 mL phosphoric acid/1L mobile phase. The flow rate was 1.5 mL min^{-1} and the detection wavelength was 230 nm. Each sample was analysed three times and relative standard deviation was 2.6%.

3. Results and Discussion

3. 1. Equilibrium Solubilities

The results of solubility measurements of carnosic acid and carnosol from rosemary extract are presented in Table 1. Each data point represents the average of a least two measurements and relative standard deviation between measurements ranged from 3.4 to 9.5%. The solubility of carnosic acid in CO_2 (Figure 1) is in the range from 3.55 10^{-2} mg/g CO_2 at 60 °C and 60 bar to 20.06 10^{-2} mg/g CO_2 at 80 °C and 402 bar. In general, the solubility of carnosic acid increases with increasing pressure, while the effect of temperature is not significant. At pressures below 200 bar (below crossover region), the solubility decreases slightly with increasing temperature. With further increase of pressure above 300 bar (above crossover region), the solubility starts to increase with increasing temperature. The comparison of measured solubility data of carnosic acid in CO_2 with the data reported by Cháfer and coworkers[26] are presented in Figure 1. Cháfer and coworkers measured the solubility of carnosic acid in CO_2 and ethanol as co-solvent using a dynamic method. They used a commercial standard of carnosic acid of purity 99%. It is well known, that by using a co-solvent, the solubility of a polar solute in CO_2 is generally higher. However, the solu-

bility data reported by Cháfer and coworkers are generally somewhat lower, except at 40 °C and above 340 bar, where they are much higher. The differences in both sets of data can be explained by different experimental methods used for solubility measurements as well as different starting material, which is probably the most important reason.

The natural rosemary extract which was applied in present study contained 73.9% of carnosic acid, 14.7% of carnosol and 11.4% of other "impurities", which probably acted sinergisticaly.

The most important conclusions of both research works, however, is that the solubility of carnosic acid in CO_2 (or CO_2 and ethanol) starts to increase significantly above 300 bar.

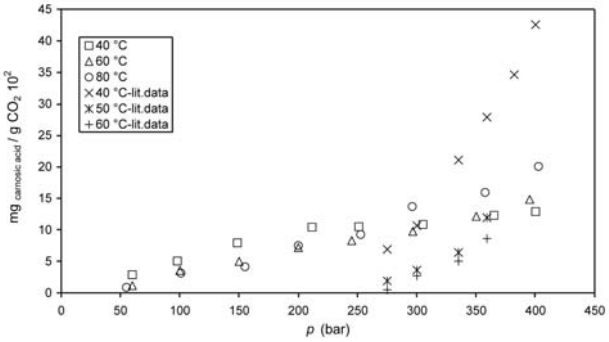

Figure 1. Solubility of carnosic acid from rosemary extract in CO_2: comparison with the literature data from Cháfer and coworkers.[26]

Figure 2 presents the solubilities of carnosic acid in CO_2 vs. density of solvent. The solubility increases with increasing solvent density at constant temperature as well as with increasing temperature at constant density, however, above density of 600 kg/m^3.

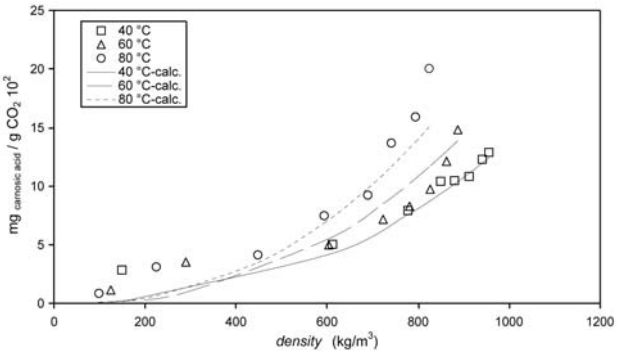

Figure 2. Solubilities of carnosic acid from rosemary extract in CO_2 (points are experimental values and lines are calculated values).

The experimentally determined solubility data of carnosic acid in CO_2 were correlated with the model proposed by Chrastil[32], where the concentration of a solute in a gas c (g/L) is related to the density of the solvent ρ (g/L) by the following equation:

$$\ln(c) = k \cdot \ln(\rho) + \frac{a}{T} + b \tag{1}$$

The constant k is obtained from the slope of a plot $\ln(c)$ versus $\ln(\rho)$ at constant temperature. The $\ln(c)$ is a linear function of $1/T$ at constant density and has a slope given by a. The value of constant b was chosen to minimize the deviation of the model from experimental data.

It was observed that solubility isotherms of carnosic acid in CO_2 are parallel in the plot $\ln(c)$ vs $\ln(\rho)$ at densities above critical density of CO_2 (ρ_c = 467.5 kg/m^3) and the equation obtained for this region is:

$$\ln(c) = 3.3936 \cdot \ln(\rho) - \frac{1510.76}{T} - 20.596 \tag{2}$$

The agreement of the model with the experimental data can be seen from Figure 2. Average absolute relative deviation (AARD) of the model from experimental data (equation 3) at densities above ρ_c solvent critical point is 5.9% at 40 °C, 14.4% at 60 °C and 13.8% at 80 °C.

$$AARD\% = \frac{100}{N} \cdot \sum_{i=1}^{N} \frac{\left| c_{i,cal} - c_{i,\exp} \right|}{c_{i,\exp}} \tag{3}$$

The amount of carnosol detected in samples taken from the autoclave and expressed in mg carnosol/g CO_2 are presented in Table 1 and Figure 3. The results show that at pressures below 300 bar, no carnosol was detected in samples taken from autoclave. At pressures above 300 bar the content of carnosol in samples vary between 0.57 10^{-2} and 2.45 10^{-2} mg/g CO_2. Furthermore, in the investigated pressure range experimental solubilities are higher at higher temperatures.

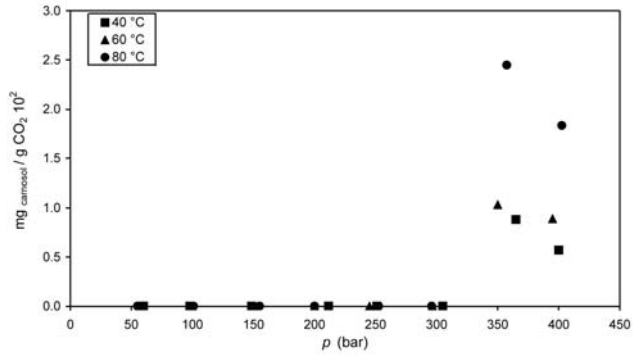

Figure 3. Concentration of carnosol from rosemary extract (73.9% carnosic acid and 14.7% carnosol) in CO_2.

The distribution coefficients of carnosol and carnosic acid (Figure 4) between the light and the heavy phase on a solvent free weight fraction basis (w_i) were calculated as:

Table 1. Solubility of carnosic acid and carnosol from rosemary extract in CO_2.

	P (bar)	ρ CO_2 (kg/m^3)[a]	$mg_{carnosic\ acid}$/g CO_2 10^2	$mg_{carnosol}$/g CO_2 10^2
40 °C				
	98	612.11	5.05	0
	148	777.85	7.93	0
	211	850.11	10.42	0
	251	880.17	10.49	0
	305	912.59	10.84	0
	365	941.52	12.29	0.88
	400	956.07	12.90	0.57
60 °C				
	100	289.95	3.55	0
	150	604.09	5.00	0
	200	723.68	7.19	0
	245	781.41	8.29	0
	297	827.08	9.76	0
	350	862.94	12.14	1.03
	395	887.63	14.83	0.89
80 °C				
	101	225.02	3.12	0
	155	448.22	4.14	0
	200	593.89	7.49	0
	252	689.76	9.25	0
	296	741.58	13.70	0
	357	794.58	15.92	2.45
	402	824.70	20.06	1.83

[a] Calculated with NIST Chemistry WebBook software.[33]

$$K_i = \frac{w_i^{light\ phase}}{w_i^{heavy\ phase}} \quad (4)$$

Generally, when the distribution coefficient of a compound is higher than 1, as in the case of carnosoic acid, then the compound is enriched in the light phase during semicontinuous extraction. Oppositelly compounds with distribution coefficient lower than 1, as in the case of carnosol, are enriched in the heavier phase.

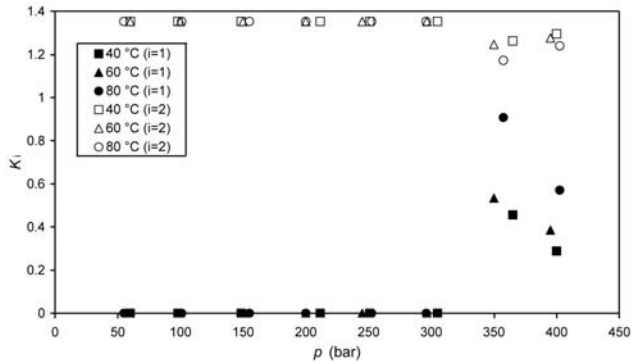

Figure 4. Distribution coefficients for carnosol (K_1) and carnosic acid (K_2) in CO_2 rich phase expressed on a solvent free basis.

4. Conclusions

The knowledge of phase equilibria in supercritical phase is essential for the design and optimization of high pressure separation process for isolation and fractionation of diterpene phenols. Natural rosemary extract containing 73.9% carnosic acid and 14.7% carnosol was investigated and solubilities in supercritical CO_2 were measured in pressure range from 98 to 402 bar and at temperatures of 40, 60 and 80 °C.

It was found that up to 300 bar only carnosic acid was solubilized in CO_2, reaching up-to 13.69 10^{-2} mg carnosic acid/g CO_2. Above 300 bar, the solubility of carnosic acid starts to increase significantly and the solubility of carnosol is approximately one order of magnitude lower compared to solubility of carnosic acid.

Parameters of the Chrastil model for solubility correlations of carnosic acid in CO_2 were determined and can be applied with a satisfying agreement with experimental values at solvent densities above critical point.

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Povzetek

Rožmarinov ekstrakt je poznan kot pomemben antioksidant, ki se pogosto uporablja v prehrambeni industriji. Glavne komponente, ki mu dajejo antioksidativne lastnosti, so diterepski fenoli, med katerima sta najpomembnejša karnozolna kislina in karnozol. Veliko raziskav je osredotočenih na izolacijo karnozolne kisline, predvsem zaradi njenega širokega spektra delovanja in ugodnih učinkov na človeško zdravje.

Namen raziskav je bil določiti topnosti karnozolne kisline in karnozola v superkritičnem CO_2. Za raziskave smo uporabili rožmarinov ekstrakt s 73.9 % karnozolne kisline in 14.7 % karnozola. Topnosti smo določili pri temperaturah 40, 60 in 80 °C in v tlačnem območju od 98 do 402 bar z uporabo statične analitične metode. Podatke ravnotežnih topnosti smo nato korelirali z empiričnim modelom po Chrastil-u.