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Solubility of red pepper (*Capsicum annum*) oil in near- and supercritical carbon dioxide and quantification of capsaicin

Kyung-Tae Kwon*, Md. Salim Uddin*, Go-Woon Jung*, Jeong-Eun Sim*, Seung-Mi Lee*, Hee-Chul Woo, and Byung-Soo Chun*,†**

*Department of Food Science and Technology, Pukyong National University, Busan 608-737, Korea
**Department of Chemical Engineering, Pukyong National University, Busan 608-737, Korea
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Abstract−Red pepper oil was extracted using near- and supercritical carbon dioxide. Extraction was carried out at pressures ranging from 10 to 35 MPa and temperatures from 30 to 60 °C, with a CO_2 flow rate of 24.01 g/min using a semi-continuous high-pressure extraction apparatus. The duration for extraction was 2 h. The highest oil yield was found at high pressure and temperature. The highest solubility of oil (1.18 mg/g of CO_2) was found at 35 MPa and 60 °C. The solubility data of red pepper oil in near- and supercritical CO_2 were fitted in Chrastil model. The fatty acid composition of red pepper oil was analyzed by gas chromatography (GC). Linoleic acid was found to be the major fatty acid in the oil. Capsaicin was quantified in different extracts by high performance liquid chromatography (HPLC). The highest capsaicin yield was found at 35 MPa and 60 °C.

Key words: Red Pepper Oil, Near- and Supercritical Carbon Dioxide, Capsaicin, Solubility

INTRODUCTION

Plants of the genus *Capsicum* (e.g., bell pepper and chili pepper), belonging to the botanical family of Solanaceae, are among the oldest cultivated plants. Their fruits have been used as spice for over 6000 years [1]. *Capsicum annum* is the most widely cultivated pepper in the world and practically all of the commercially available fresh, processed, dried and frozen peppers belong to this species. *Capsicum* fruits are used throughout the world because of their unique flavor and pungency and also have widespread use as a drug [2]. Pungency, a quality criterion in chili, is caused by a group of vanillylamides, the capsaicinoids. Capsaicinoids are synthesized in the placenta of the fruits by an enzymatic condensation of vanillylamine and medium chain length fatty acids [3]. More than 20 capsaicinoids, differing only in the fatty acid structures, have been described [4]. Capsaicin (8-methyl-*N*-vanillyl-6-noneamid) is the most pungent of a group of compounds called capsaicinoids that can be isolated from hot peppers (*Capsicum annuum* L.). Capsaicin and dihydrocapsaicin, which are responsible for 90% of the total pungency, are the most abundant principles of hot peppers. Among the capsaicinoids, capsaicin comprises more than 70% pungency of red pepper [5,6].

Capsaicin is important in foods due to its hot flavor. Topically applied capsaicin is also useful in alleviating the pain associated with diabetic neuropathy, osteoarthritis and psoriasis [7,8]. Capsaicinoids act on a receptor sensing noxious stimuli, the vanilloid type 1 receptor TRPV1, being a member of the transient receptor potential family of cation channels [9,10].

Several research works have been carried out to identify and quantify the capsaicinoids from different variety of pepper [4,11-13]. In most cases, capsaicin is extracted using conventional organic solvents that are harmful to human health as well as the environment. Beside this, the production of plant extracts is currently limited by safety and regulatory constraints to the concentration of toxic residues of conventional organic solvents [14]. Supercritical fluid extraction has been widely employed as an alternative to organic solvent for the extraction of a variety of compounds from different matrices [15-21]. Carbon dioxide (CO_2) is probably the most widely used supercritical fluid because of its critical temperature (31.1 °C), which makes it an ideal solvent for extracting thermally labile materials. It is also excellent as a solvent due to its inertness, non-toxicity, non-flammability and low cost. CO_2 is applied in supercritical fluid extraction processes at near-environmental temperatures, thus minimizing heat requirement and thermal damage to bioactive compounds [22]. Therefore, the objectives of this study were to extract red pepper oil, to measure the solubility of the oil in near- and supercritical CO_2 and to determine the fatty acid composition and capsaicin content of the oil at different extraction temperature and pressure.

EXPERIMENTAL

1. Materials

The matured (red color) pepper was collected from Busan area, Republic of Korea. Capsaicin was purchased from Sigma-Aldrich, St. Luis, Mo., USA. Carbon dioxide (99.99% pure) was supplied by KOSEM, Korea. All other reagents were of analytical or HPLC grade.

2. Sample Preparation

Red pepper sample was dried in an oven at low temperature for about 48 h. The dried sample was mechanically crashed and sieved in 500 μm by mesh. The sieved sample was used for near- and supercritical CO_2 extraction of oil.

3. Near- and Supercritical CO_2 Extraction

The laboratory scale near- and supercritical fluid extraction system, shown in Fig. 1, can be operated at pressure up to 35 MPa.

†To whom correspondence should be addressed.
E-mail: bschun@pknu.ac.kr

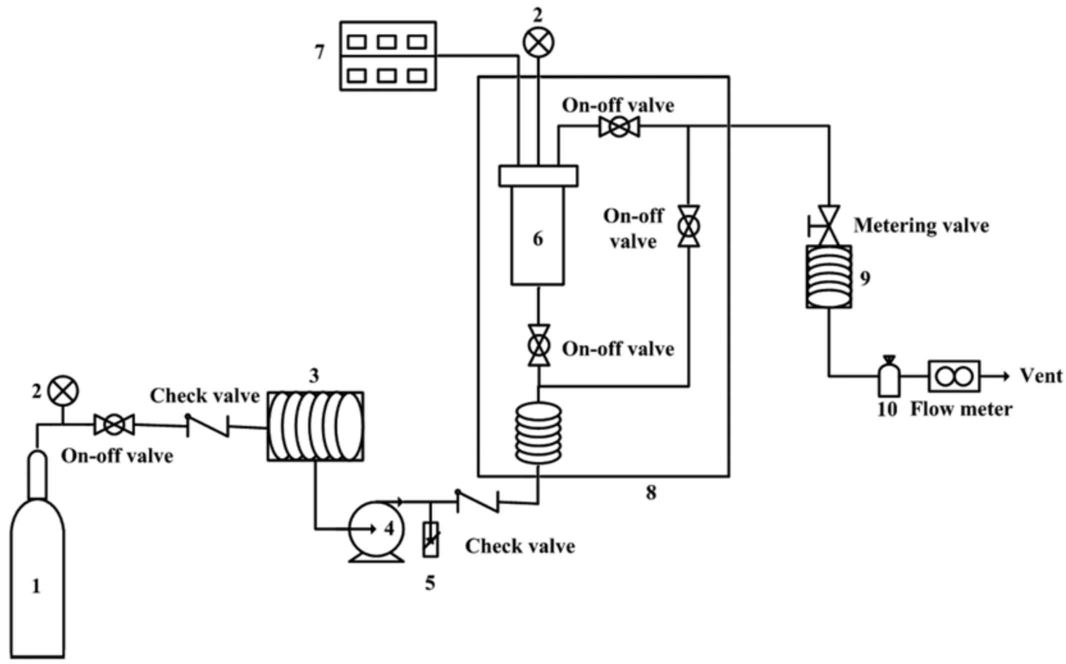

Fig. 1. Schematic diagram of near- and supercritical CO_2 extraction process.

1. CO_2 tank	3. Chiller	5. Safety valve	7. Temperature indicator	9. Water bath
2. Pressure gauge	4. Pump	6. Extractor	8. Electric voen	10. Separater

Twelve grams of dried red pepper sample was loaded into a 50 mL stainless steel extraction vessel. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with a cap another layer of cotton was used at the top of the sample. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump up to the desired pressure. A digital pressure controller was used to maintain the pressure of CO_2 accurately. The extractor was placed into an electric oven for maintaining the extraction temperature. The applied pressure and temperature ranges for near- and supercritical CO_2 extraction were 10-35 MPa and 30-60 ℃ at a constant extraction time of 2 h. The flow rate of CO_2 was constant at 24.01 g/min for all extraction conditions. Flow rates of CO_2 passing through the apparatus were measured with a gas flow meter. The oil extracted by near- and supercritical CO_2 was collected by a glass separating vessel. The amount of extract obtained at regular intervals was established by weight using a balance with a precision of ±0.001 g. The extracted oil was then stored at –40 ℃ until further analysis.

4. GC Analysis for Fatty Acid Compositions

The fatty acid compositions of pepper oil obtained by near- and supercritical CO_2 extraction were determined by GC using a Hewlett Packard gas chromatograph (5890 Series II GC system). The fatty acid methyl esters were prepared firstly according to AOCS official method Ce 2-66 [23] and then separated using an Agilent DB-Wax capillary column (30 m length×0.250 mm internal diameter, 0.25 µm of film). Nitrogen was used as a carrier gas (1.0 mL/min) of fatty acid methyl esters. The oven temperature was programmed starting at a constant temperature of 130 ℃ for 3 min and then increased to 240 ℃ at a rate of 4 ℃/min and held at 240 ℃ for 10 min. Injector and detector temperatures were 250 ℃. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, USA).

5. Determination of Capsaicin by HPLC

Capsaicin measurement was carried out using a Waters HPLC equipped with a model 600E system controller, a model 484 UV/VIS detector and an Eclipse Plus C18 column (5 µm, 4.6×250 mm, Agilent, USA). Capsaicin was quantified by an isocratic method reported by De la Fuente [24]. A mobile phase consisting of acetonitrile and water at the volume ratio of 70 : 30 was eluted at the flow rate of 1 mL/min. Capsaicin was detected at the wavelength of 280 nm. The amount of capsaicin in the extract was measured based on the peak area of the standard capsaicin.

RESULTS AND DISCUSSION

1. Near- and Supercritical CO_2 Extraction of Oil

Near- and supercritical fluid extraction curves of red pepper oil at different pressures (10-35 MPa) and temperatures (30-60 ℃) are shown in Fig. 2(a)-(f). The highest oil yield obtained by supercritical CO_2 extraction was 0.08 g/g of red pepper at 35 MPa and 60 ℃. The applied pressure and temperature variation greatly affected the oil solvating power of near- and supercritical CO_2. At constant temperature, the solubility of oil in near- and supercritical CO_2 increased with the increase in pressure. This happened due to direct increase of density and hence solvating power of near- and supercritical CO_2. The effect of pressure can be attributed to the increase in solvent power by strengthening intermolecular physical interactions [25-27]. Similar pressure effect was reported in supercritical CO_2 extraction of sesame seed [21] and green coffee oil [28]. At subcritical CO_2 extraction, the applied temperature (30℃) was very near to the critical temperature of CO_2. However, at this temperature, subcritical CO_2 showed almost similar trend of extraction by supercritical CO_2 at

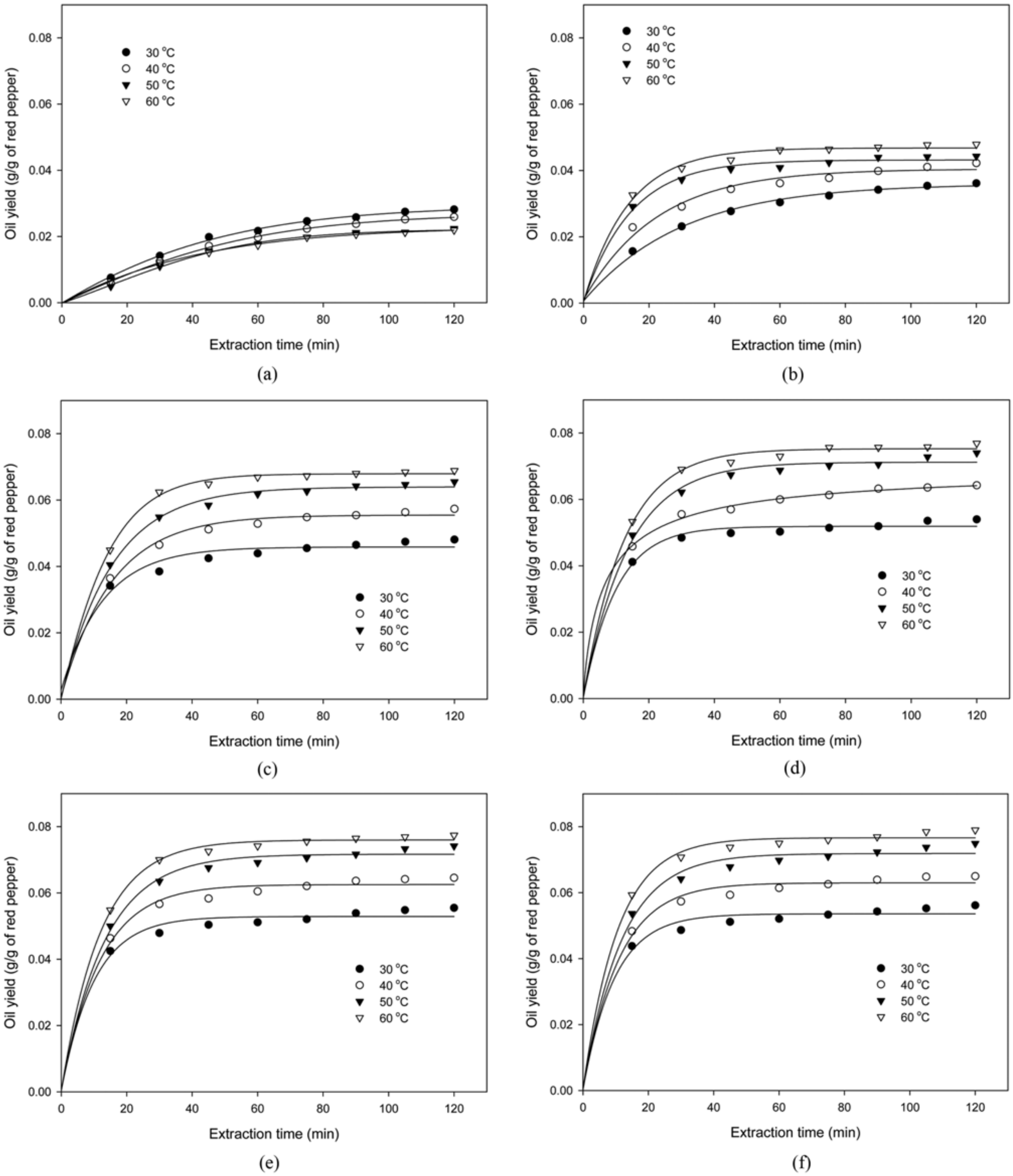

Fig. 2. (a)-(f) Near- and supercritical CO_2 extraction of red pepper oil at different pressures and temperatures. (a) 10 MPa (b) 15 MPa (c) 20 MPa (d) 25 MPa (e) 30 MPa and (f) 35 MPa.

different pressures.

At constant pressures of 15, 20, 25, 30 and 35 MPa, the yield increased with the increase in temperature. Due to increase in temperature, the solvent density decreased. However, despite the decrease in solvent density, the oil yield increased with temperature which can be attributed to the increase of oil components vapour pressure. The effect of increase of solute vapor pressure seems to have dominated over solvent density. Azevedo et al. [28] reported similar effect of supercritical CO_2 extraction of green coffee oil. In contrast, at a constant pressure of 10 MPa, the oil yield decreased with the increase in temperature. In this extraction condition, the decrease of solvent density seems to have dominated over solute vapour pressure.

Table 1. Solubility of red pepper oil by near- and supercritical CO_2 at different pressures and temperatures

Temperature (°C)	Pressure (MPa)	Solubility (mg oil/g CO_2)		
		Red pepper	Black currant seed[a]	Apricot seed[b]
30	10	0.24		
	15	0.38		
	20	0.64		
	25	0.79		
	30	0.80		
	35	0.81		
40	10	0.21		
	15	0.48	1.3	1.1
	20	0.77	3.0	
	25	0.92		
	30	0.94		
	35	0.96		
50	10	0.18		
	15	0.62	0.59	0.9
	20	0.91	2.4	
	25	1.03		
	30	1.06		
	35	1.07		
60	10	0.2		
	15	0.67		
	20	1.04		
	25	1.15		
	30	1.17		
	35	1.18		

[a]Sovova et al. [28]
[b]Ozkal et al. [29]

It was also found that the extraction curves reached a plateau within the time ranges of 30-60 min in different extraction conditions. At high pressure, the extraction of oil by near- and supercritical CO_2 was faster compared to low pressure. It agrees with a generalized finding that oil yield increases with the increase in solvent density.

2. Solubility Measurement of Red Pepper Oil

The oil solubility at each extraction condition of temperature and pressure was obtained from the slope of the linear section of the extraction curves shown in Fig. 2(a)-(f). Table 1 shows the oil solubility of red pepper oil at different extraction conditions. The highest solubility value was 1.18 mg/g of CO_2 at 35 MPa and 60 °C. Solubility of oil depends on the density of CO_2 and vapour pressure of oil components as described in near- and supercritical CO_2 extraction section. At similar pressure and temperature, Sovova et al. [29] and Ozkal et al. [30] reported higher solubility of oil from black currant and apricot seeds, respectively. The variation of solubility might be due to variation of sample, extraction unit, sample size, CO_2 flow rate and so more.

3. Fatty Acid Compositions

Red pepper oil was characterized by a reddish brown color and a characteristics pungent odor. The fatty acid composition of the oil obtained from red pepper by near- and supercritical CO_2 extraction is shown in Table 2. It is evident from the data that the fatty acid composition remarkably changed at different extraction conditions. Red pepper oil contained higher amount of palmitic, oleic and linoleic acid. Among the unsaturated fatty acid, linoleic acid was predominant at all extraction conditions, and it was more than 40% of total fatty acid content of red pepper oil. The highest percentage of linoleic acid (63.30%) was found at 35 MPa and 40 °C. In contrast, among the saturated fatty acids, palmitic acid was present in the highest amounts. Red pepper oil also contained significant amount of stearic and linolenic acid.

4. Capsaicin Yield

Capsaicin yield obtained by near- and supercritical CO_2 extraction at different pressures and temperatures is shown in Fig. 3. The highest yield of capsaicin was 3.59 mg/g of red pepper powder at 35 MPa and 60 °C. It was found that the yield increased with the increase in pressure up to 35 MPa at 50 and 60 °C. The increased pressure caused higher density of fluid resulting in higher yield of capsaicin. Gnayfeed et al. [31] also reported that capsaicin yield increased with the increase in pressure. However, at 30 and 40 °C the highest yield was achieved at 20 MPa. This dual behavior of yield due to increasing pressure can be explained by a double effect of increased density of supercritical CO_2 and decreased diffusion coefficient. Since increased pressure also increased the density of the supercritical fluid that is associated with an increase in its solvating power, in this way it enhanced the extraction process. On the other hand, the decreased diffusivity caused a reduced interaction between the supercritical fluid and matrix resulting in the decreased yield by extraction process. At 30 and 40 °C, and 20 MPa, the decrease in diffusion coefficient was more dominant than solvating power, resulting in high yield of capsaicin. Careri et al. [32] reported similar effect for carotenoid extrac*tion from algae.*

The effect of temperature on capsaicin yield can be explained by a complex balance of the decreased density of fluid and the increased vapor pressure of solute. At 15 and 20 MPa, the highest yield was obtained at 40 °C. In contrast, for the operating pressures of 25, 30 and 35 MPa, the highest yield was found at 60 °C. Increasing the temperature decreased the fluid density, but increased the solute vapor pressure, which enhanced the yield of extraction process. At 25, 30 and 35 MPa, the solute solubility was higher due to the increased vapor pressure of capsaicin. At 15 and 20 MPa, this effect prevails up to 40°C. Similar temperature effects on solute solubility were reported by Careri et al. [32] and Lopez et al. [33]. At 10 MPa, the capsaicin yield decreased with the increase in temperature. This result was directly related to the density of supercritical fluid because at low pressure the density of fluid decreased with the increase in temperature.

5. Solubility Correlation

To correlate the solubility of red pepper oil in the near- and supercritical CO_2, the Chrastil [34] model was adopted. It proved to be useful in correlating vegetable oils solubility [29,35]. This model is based on the direct relationship between solubility and density of a solvent. The correlations based on empirical density are very useful to determine the solubility of solids and liquids in compressed fluids, as they are both simple and do not require physicochemical properties of the solute. Despite its limitations, the usefulness of the equation has been proven in supercritical fluid extraction work and is easy to use. The experimental values represented by points and the calculated solubilities (lines) are shown in Fig. 4, where the iso-

Table 2. Fatty acid compositions of red pepper oil obtained at different extraction conditions

Pressure (MPa)	Temperature (ºC)	Fatty acids				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
10	30	15.17	2.81	13.60	55.88	3.89
	40	17.69	2.16	13.39	58.41	4.00
	50	13.91	1.96	12.84	56.16	3.02
	60	14.44	2.69	14.61	58.12	3.10
15	30	16.08	2.92	14.00	56.82	3.03
	40	16.98	2.19	13.97	58.40	3.48
	50	15.51	2.12	13.32	51.92	3.36
	60	16.84	2.27	14.13	50.97	3.71
20	30	14.44	2.06	13.13	58.70	3.81
	40	16.51	2.22	14.19	59.69	3.53
	50	15.90	2.41	11.58	60.56	3.32
	60	17.09	2.19	14.22	53.47	3.60
25	30	13.19	1.89	10.28	46.70	2.54
	40	14.35	1.97	11.20	51.52	2.80
	50	14.75	1.98	11.12	48.48	3.15
	60	13.29	1.78	10.16	45.81	2.71
30	30	16.65	2.12	13.51	59.97	2.57
	40	17.12	2.18	13.71	63.18	3.12
	50	17.03	2.21	13.74	63.29	3.05
	60	16.81	2.22	13.90	62.97	3.43
35	30	16.55	2.18	13.58	59.90	2.68
	40	16.93	2.25	13.96	63.30	3.03
	50	17.39	2.29	13.90	62.99	3.12
	60	16.93	2.16	14.04	63.09	3.68

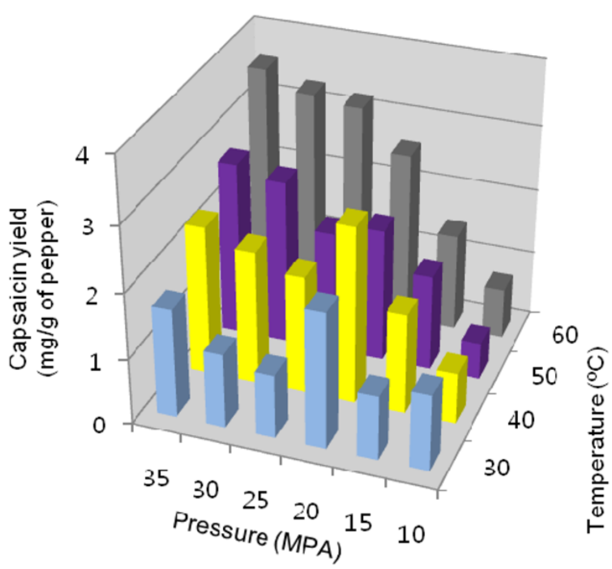

Fig. 3. Capsaicin yield in red pepper oil by near- and supercritical CO_2 extraction at different pressures and temperatures.

therms and the effects of temperature and solvent density are clearly shown. The correlation of oil solubility with solvent density was obtained from the Eq. (1).

$$y = \rho_{CO_2}^{k} \exp\left(\frac{a}{T} + b\right) \quad (1)$$

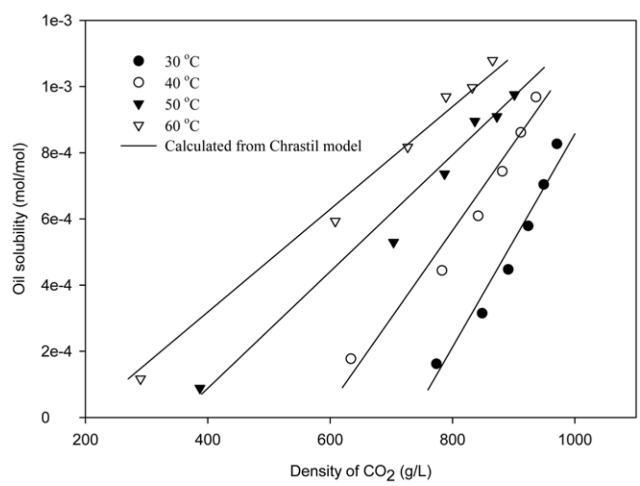

Fig. 4. Experimental data of red pepper oil solubility as a function of CO_2 density and calculated results from Chrastil model.

where y is the solubility of red pepper oil (mol/mol), ρ_{CO_2} is the density of CO_2, T is experimental temperature (K) and a, b and k are empirical fitting parameters. The solubility data of red pepper oil were fitted well in the Chrastil model because at a given temperature, almost a linear relation between solubility of oil and solvent density was obtained. Daood et al. [36] also reported that the solubility of pungent spice paprika oil in supercritical CO_2 was fitted in the Chrastil model.

CONCLUSIONS

Red pepper oil was extracted in a high pressure apparatus using near- and supercritical CO_2 at different pressures and temperatures. The highest yield of oil was found at 35 MPa and 60 °C. The solubility of oil calculated from extraction curve was also higher at high pressure and temperature. Red pepper oil contained highest amount of linoleic acid in all extraction conditions, and it was significantly higher than that of other fatty acids content. The highest capsaicin yield obtained by supercritical CO_2 extraction was 3.59 mg/g of red pepper at 35 MPa and 60 °C. The identification of species/cultivars with high level of capsaicin, as well as tracing of biological activities, is important for human and other living organisms. Therefore, the determination of capsaicin and fatty acids in supercritical CO_2 extract might be helpful for their proper use in biological research.

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REFERENCES

1. L. Perry, R. Dickau, S. Zarrillo, I. Holst, D. M. Pearsall, D. R. Piperno, M. J. Berman, R. G. Cooke, K. Rademaker, A. J. Ranere, J. S. Raymond, D. H. Sandweiss, F. Scaramelli, K. Tarble and J. A. Zeidler, *Science*, **315**, 986 (2007).
2. A. A. Newman, *Chem. Prod. Chem. News*, **16**, 413 (1953).
3. T. Suzuki and K. Iwai, in *The alkaloids: Chemistry and pharmacology*, A. Brossi, Eds., Academic Press, Orlando, FL, USA, **23**, 227 (1984).
4. R. Thiele, E. Mueller-Seitz and M. Petz, *J. Agric. Food Chem.*, **56**, 4219 (2008).
5. K. Iwai, T. Suzuki and H. Fujiwake, *J. Chromatogr.*, **172**, 303 (1979).
6. S. Kosuge and M. Furata, *Agric. Biol. Chem.*, **34**, 248 (1970).
7. W. Y. Zhang and A. L. W. Po, *Eur. J. Clin. Pharmacol.*, **46**, 517 (1994).
8. M. Majeed, V. Badmev, L. Prakash, S. Natrajan and S. Gopinathan, *Capsaicin: The anti-arthritic phytochemical*, Nutri. Science Publ. Inc., Piscataway, NJ, USA (1977).
9. M. Tominaga, *Chem. Senses.*, **30**(Suppl. 1), i191 (2005).
10. S. E. Jordt, D. D. McKemy and D. Julius, *Curr. Opin. Neurobiol.*, **13**, 487 (2003).
11. A. Saria, F. Lembeck and B. Shofitaheh, *J. Chromatogr.*, **208**, 41 (1981).
12. A. M. Krajewska and J. J. Powers, *J. Assoc. Off Anal. Chem.*, **70**, 926 (1987).
13. J. Yao, M. G. Nair and A. Chandra, *J. Agric. Food Chem.*, **42**, 1303 (1994).
14. N. Sanders, in *Extraction of natural products using near-critical solvents*, M. B. King, T. R. Bott Eds., Blackie Academic & Professional, London, UK, 34 (1993).
15. K. Y. Kang, D. H. Ahn, G. T. Wilkinson and B. S. Chun, *Korean J. Chem. Eng.*, **22**, 399 (2005).
16. P. Bhattacharjee, R. S. Singhal and S. R. Tiwari, *J. Food Eng.*, **79**, 892 (2007).
17. S. Hamdan, H. G. Daood, M. T. Markus and V. Illes, *J. Supercrit. Fluids*, **44**, 25 (2008).
18. K. P. Yoo, H. Y. Shin, M. J. Noh and S. S. You, *Korean J. Chem. Eng.*, **14**, 341 (1997).
19. M. J. Noh, E. S. Choi, S. H. Kim, K. P. Yoo, Y. H. Choi, Y. W. Chin and J. W. Kim, *Korean J. Chem. Eng.*, **14**, 109 (1997).
20. K. W. Chan and M. Ismail, *Food Chem.*, **114**, 970 (2009).
21. M. P. Corso, M. R. Fagundes-Klen, E. A. Silva, L. C. Filho, J. N. Santosc, L. S. Freitas and C. Dariva, *J. Supercrit. Fluids*, **52**, 56 (2010).
22. G. Brunner, Springer, New York, USA (1994).
23. AOCS, American Oil Chemists' Society, Champaign, Illinois, USA (1998).
24. J. C. De la Fuente, O. Jose, Valderrama, S. B. Bottini and J. M. del Valle, *J. Supercrit. Fluids*, **34**, 195 (2005).
25. A. Morita and O. Kajimoto, *J. Phys. Chem.*, **94**, 6420 (1990).
26. S. Bai, M. V. Craig, L. F. Liu, C. L. Mayne, R. J. Pugmire and D. M. Grant, *J. Phys. Chem.*, **101**, 2923 (1997).
27. D. S. Bulgarevich, T. Sako, T. Sujeta, K. Otake, Y. Takebayashi, C. Kamizawa, Y. Horikawa and M. Kato, *Ind. Eng. Chem. Res.*, **41**, 2074 (2002).
28. A. B. A. de Azevedo, T. G. Kieckbush, A. K. Tashima, R. S. Mohamed, P. Mazzafera and S. A. B. Vieira de Melo, *J. Supercrit. Fluids*, **44**, 186 (2008).
29. H. Sovova, M. Zarevucka, M. Vacek and K. Stransky, *J. Supercrit. Fluids*, **20**, 15 (2001).
30. S. G. Ozkal, M. E. Yener and L. Bayindirli, *Int. J. Food Sci. Technol.*, **41**, 399 (2006).
31. M. H. Gnayfeed, H. G. Daood, V. Illes and P. A. Biacs, *J. Agric. Food Chem.*, **49**, 2761 (2001).
32. M. Careri, L. Furlattini, A. Mangia, M. Musci, E. Anklam, A. Theobald and C. V. Holst, *J. Chromatogr. A*, **912**, 61 (2001).
33. M. Lopez, L. Arce, J. Garrido, A. Rios and M. Valcarcel, *Talanta*, **64**, 726 (2004).
34. J. Chrastil, *J. Phys. Chem.*, **86**, 3016 (1982).
35. P. Maheshwari, Z. L. Nikolov, T. M. White and R. Hartel, *J. Am. Oil Chem. Soc.*, **69**, 1069 (1992).
36. H. G. Daood, V. Illes, M. H. Gnayfeed, B. Meszaros, G. Horvath and P. A. Biacs, *J. Supercrit. Fluids*, **23**, 143 (2002).