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Rapid and Efficient Extraction of Curcumins from Curry Powder Using Supercritical CO_2

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A dried curry powder is well known food additive in many Asian countries. Curcumins are main coloring substances and active compounds in curry powder which is the natural yellow pigment in turmeric isolated from the rhizome of the plant *Curcuma longa*. Curcumins are consisted of curcumin ([(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) and two related compounds, demethoxy-curcumin (DMC) and bis demethoxycurcumin (BDMC). The chemical structure of curcumin is shown in Figure 1. Curcumins gives specific flavor and yellow color to curry powder.[1] Curcumins was found to inhibit the generation of reactive oxygen species including superoxide dismutase and hydrogen peroxide in peritoneal macrophages.[2] Curcumins also inhibits lipo-polysaccharide and interferon-γ-induced production of nitric oxide in macrophages[3] and inhibition of inducible nitric oxide synthase gene expression in isolated mouse peritoneal macrophages.[4] Curcumins have also been reported to exhibit anti-clastogenic,[5] anti-fungal[6] and anti-viral properties.[7]

For the extraction of useful organic compounds from natural products, supercritical fluid extraction (SFE) has been currently used. Since the solubilities[8] of curcumins in supercritical CO_2 and ethanol modified supercritical CO_2 are fairly high (6.51% wt/wt in supercritical CO_2, 22.58% wt/wt in ethanol modified supercritical CO_2 at 30 Mpa, 318 K), supercritical fluid extraction can be one of the most effective extraction method for the extraction of curcumins. Until recently little information has been available in the literature regarding selective extraction of curcumins from curry powder, although essential oils including curcumins have been extracted using supercritical fluid.[8,9] In this paper, a new method has been developed to extract curcumins selectively from curry powder. This new method showed much higher extraction efficiency than the method described in recent Korean Patent.[10]

Extraction with supercritical fluids as solvents have received wide attention recently. A number of potential advantages including rapid extraction time, minimized sample handling steps, more efficient extraction, increased selectivity are possible with supercritical fluid extraction.

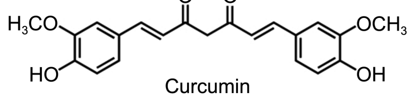

Figure 1. Chemical structure of curcumin.

These advantages of SFE accrue from the properties of a solvent at temperatures and pressures above its critical point. At elevated pressure, this single phase will have properties that are intermediate between those of the gas and the liquid phases and are dependent on the fluid composition, pressure, and temperature. The compressibility of supercritical fluids is large just above the critical temperature, and small change in pressure result in large changes in the density of the fluid.[10] The density of a supercritical fluid is typically 10^2-10^3 times that of gas. Consequently, molecular interactions increase due to shorter intermolecular distances. However, the diffusion coefficients and viscosity of the fluid, although density dependent, remain more like that of a gas. The "liquidlike" behavior of a supercritical fluid results in enhanced solubilizing capabilities for the less polar compounds. These properties allow similar solvent strengths to liquids but with greatly improved mass-transfer properties which provide the potential for more rapid extraction rates and more efficient extraction due to better penetration of the matrix.

Among a wide variety of supercritical fluids, CO_2 has been the most commonly employed due to its comparatively low critical temperature (31.1 °C) and pressure (78.3 atm) together with its another advantages such as environmental acceptance and non toxicity to human health.[11]

As examples of supercritical fluid extraction, Sugiyama *et al.*[12] was successfully performed to extract caffeine from the green beans and Schneiderman *et al.*[13] was successfully performed to extract menadione (vitamin K_3) from animal food.

The advantages of supercritical fluid extraction of curcumin from curry powder compared conventional liquid extraction are the followings; (a) more selective extraction is possible (b) it is less expensive in terms of solvent cost and laboratory time, (c) carbon dioxide is available, to be used as a pure solvent, with its convenient critical temperature, its non-toxicity.

Experimental

Materials and Reagents. Dried and powdered curry powder was purchased from E-mart (Chunchon, Korea) and

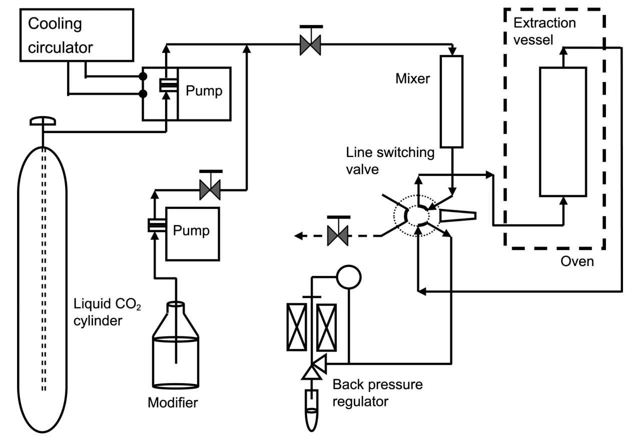

Figure 2. Schemetic flow diagram of SFE modular system.

used for supercritical fluid extraction. Carbon dioxide (99.99 wt % pure) was provided by Bakryoung Special Gas (Chunchon, Korea). All solvents were HPLC grade from Fisher Scientific Korea Ltd.

SFE System. Supercritical fluid extractions of curcumin were performed using a JASCO (Tokyo, Japan) LC-900 SFE system. The schematic diagram of the system is shown in Figure 2. This system consisted of three sections: fluid delivery, extraction, and collection. The fluid delivery section included a high pressure pump, which delivered supercritical fluid CO_2. In the extraction section, supercritical fluid extractions were performed with supercritical fluid CO_2. The collection section included a back pressure regulator, which kept the pressure of an extraction vessel at the desired value. The effluent flowing through the back pressure regulator reduced its pressure to atmospheric and thereby solutes in the effluent reduced their solubility to virtually zero. In this way, the solutes were deposited and collected in a collection vessel. Since we used pure CO_2 as a extracting solvent, the extracts were collected without solvent in the collection vessel.

HPLC Analysis. Supercritical fluid extracts of curry powder were analyzed by high performance liquid chromatography (HPLC). The HPLC system consisted of a JASCO PU-980 pump, an UV-2075 UV detector and a Waters Spherisorb S5

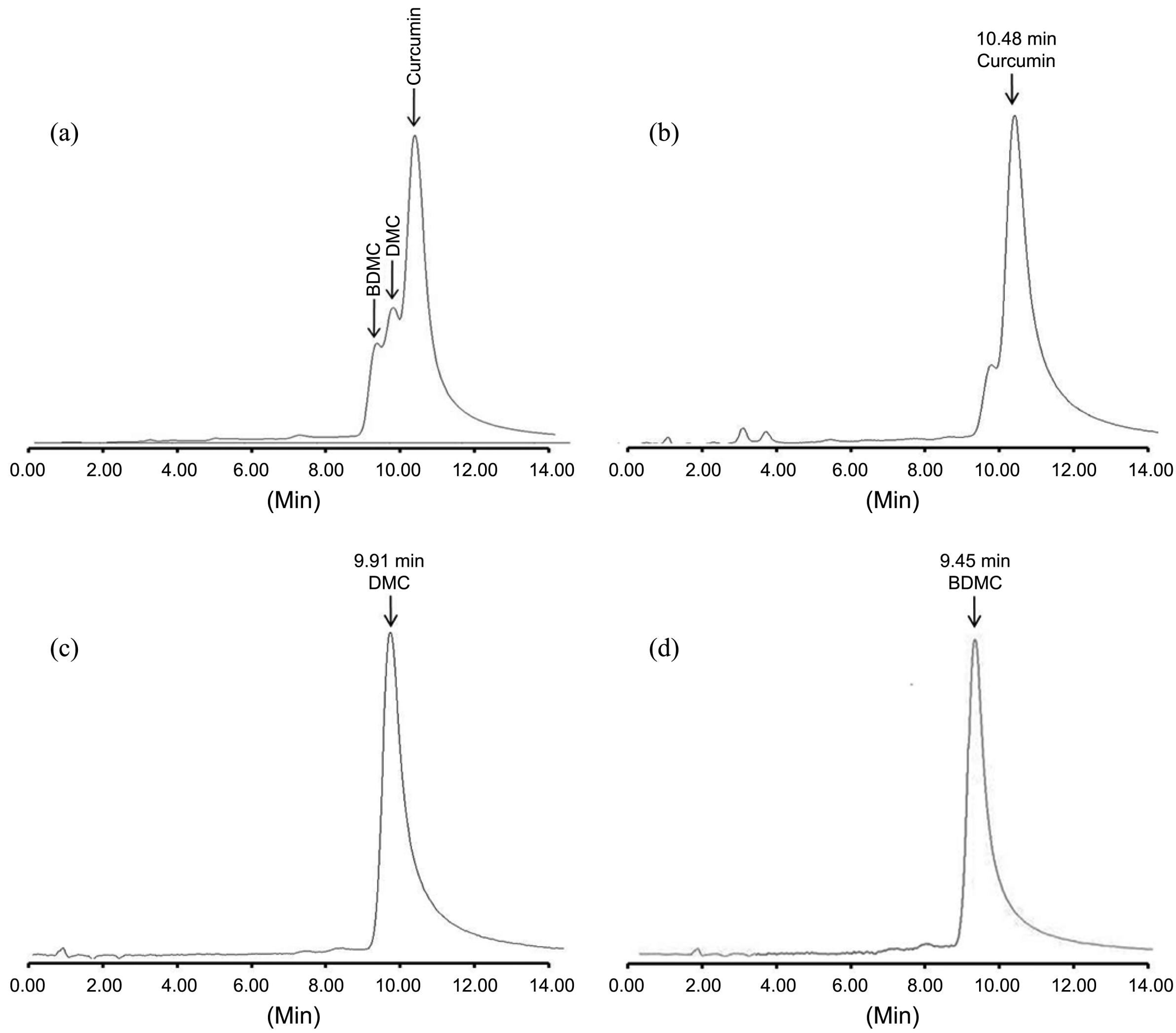

Figure 3. HPLC chromatograms of curcumins ((a) typical SFE extract from a curry powder, (b): standard curcumin, (c): standard DMC, (d): standard BDMC).

ODS2 (4.6 × 150 mm, 5 μm particle size) column. An aliquot of 10 μL was injected into the HPLC system. The mobile phase was a mixture of 30% of methanol and 70% of water. The flow rate was 0.8 mL/min, and UV detector operating at 424 nm. A typical HPLC chromatogram is shown in Figure 3.

Results and Discussion

SFE conditions for the optimization of the extraction of curcumins from a curry powder were evaluated. The amounts of extracted curcumins were quantitatively measured by HPLC analysis described in the earlier section.

Effect of Pressure on Extraction Efficiency. It is important to find the optimum SFE operating conditions which would result in the most efficient extraction of curcumins from a curry powder. In particular, the pressure and temperature of supercritical fluid, which are the most important parameters to be optimized, for the efficient SFE experiment. Extraction efficiency of curcumins was investigated as a function of extraction pressure (Table 1). Extraction pressures of 200, 225, 250 and 275 atm were chosen. The pressure of the supercritical fluid plays an important role in the SFE of curcumins from a curry powder. An increase in pressure causes an increase in the fluid density, and thus it results in the increase in the solvating power of the supercritical fluid. As shown in Table 1, the extraction efficiency increases with increasing pressure of extracting fluid until the pressure reaches 250 atm.

At higher pressure than 250 atm, the extraction efficiency decreases because extraction of curcumins can be competed with extraction of other compounds in the sample matrix. Therefore, the optimized pressure was chosen as 250 atm for the extraction of curcumins from a curry powder.

Effect of Temperature on Extraction Efficiency. To find the optimum extraction temperature, the temperature of the extraction vessel was varied from 40 °C to 70 °C, under the pressure of 250 atm (Table 2). As shown in Table 2, the best extraction efficiency was obtained at the temperature of 60 °C. From 40 °C to 60 °C. increased temperature resulted in increased extraction efficiency of curcumins. At higher temperature, curcumins become more volatile and can be easily separated from the matrix. However, at the fairly high temperature (70 °C), the extraction efficiency of curcumins was decreased due to the decreased density of supercritical fluid.

Effect of Modifier on Extraction Efficiency. The effect of modifier on the extraction efficiency of curcumins is shown in in Table 3. The modifier used in this study was ethanol which is known as the most effective modifier for the SFE of curcumins.[8] As can be seen in Table 3, the poor extraction result (4.53 mg/g) with neat supercritical CO_2 was obtained. This result is probably caused by the fact that curcumins consisit of polar fuctional groups, *i.e.* phenol and ketone. The use of modifier can have a profound effect on increasing the solubility levels of polar solutes in supercritical fluids. When the flow rate of modifier was increased from 0.1 mL/min to 0.3 mL/min, the extraction yield of curcumins increased from 14.29 mg/g to 31.07 mg/g. But at higher flow rate of modifier (0.4 mL/min), the extraction yield of curcumins was decreased. The decreased extraction of curcumins with 0.4 mL/min flow rate of modifier may be attributed to some solute-modifier interactions that can weaken the solute-supercritical CO_2 interactions. Similar results were obtained for other samples and matrices using

Table 1. Supercritical fluid extraction of curcumins using various pressures

Pressure, atm	The amount of extracted total curcumins (mg/g)
275	4.15 ± 0.4
250	4.53 ± 0.3
225	3.68 ± 0.3
200	2.55 ± 0.3

Experimental condition: 120 min extraction at 40 °C, CO_2 flow 3.0 mL/min. RSDs based on triplicate extractions under each condition.

Table 2. Supercritical fluid extraction of curcumins using various temperatures

Temperature, °C	The amount of extracted total curcumins (mg/g)
70	7.26 ± 0.4
60	8.04 ± 0.5
50	4.68 ± 0.4
40	4.53 ± 0.3

Experimental condition: 120 min extraction at 250 atm, CO_2 flow 3.0 mL/min. RSDs based on triplicate extractions under each condition.

Table 3. Supercritical fluid extraction of curcumins using different flow rates of ethanol as a modifier

Flow rate of modifier	The amount of extracted total curcumins (mg/g)
none	4.53 ± 0.3
0.1 mL/min	14.29 ± 0.5
0.2 mL/min	21.58 ± 0.5
0.3 mL/min	31.07 ± 0.6
0.4 mL/min	21.78 ± 0.6

Experimental condition: 120 min extraction at 60 °C and 250 atm, CO_2 flow 3.0 mL/min. RSDs based on triplicate extractions under each condition.

Table 4. Supercritical fluid extraction of curcumins using various extraction times

Extraction time, min	The amount of extracted total curcumins (mg/g)
90	26.45 ± 0.5
120	31.07 ± 0.6
150	31.14 ± 0.6

Experimental condition: Supercritical fluid extraction with a ethanol modified CO_2 fluid (3.0 mL/min CO_2 + 0.3 mL/min ethanol) at 60 °C and 250 atm. RSDs based on triplicate extractions under each condition.

modified CO_2.[14,15]

Effect of Extraction Time. In order to determine the optimal extraction time, the extraction time was varied from 90 min to 150 min by the interval of 30 min (Table 4). As can be seen in Table 4, when the extraction time was increased from 90 min to 120 min, the extraction yield of curcumins increased from 26.45 mg/g to 31.07 mg/g. However, no significant increase between 120 min and 150 min was made. Therefore, to have a rapid extraction of curcumins from a curry powder, we selected a shorter extraction time (120 min) as the optimum extraction time.

Comparison with Solvent Extraction. For the comparison, curcumins were extracted using the conventional solvent extraction method. The solvent used was methanol since methanol was reported as the most efficient solvent for the extraction of curcumins.[16] The extraction procedure was the same as that described in the literature.[16] The amounts of extracted curcumins were 17.82 mg/g curcumin, 4.09 mg/g DMC and 2.82 mg/g BDMC. The total amounts of extracted curcumins were 24.73 mg/g.

In conclusion, curcumins were successfully extracted with a ethanol modified supercritical CO_2 fluid (3.0 mL/min CO_2 + 0.3 mL/min ethanol) at 60 ºC and 250 atm. Under these conditions, the extracted amount of curcumins was 31.07 mg from 1 g of curry powder. In the case of using solvent extraction, only 24.73 mg of curcumins was extracted from 1 g of curry powder. The CO_2-ethanol fluid system gave a higher extraction efficiency than a solvent extraction system for the extraction of curcumins from a curry powder. By use of SFE, sample handling steps are minimized, thus reducing the possible losses of curcumins and saving analysis time. No clean-up steps are employed since the SFE with ethanol modified CO_2 gives clean extracts which can be easily analyzed with HPLC.

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