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主　論　文　の　要　旨

論文題目　**Process Integration of Supercritical Fluid with Novel Techniques in Extraction and Microencapsulation of Bioactive Compounds**
(生理活性物質の抽出とマイクロカプセル化のための超臨界流体と新規技術のプロセス複合化)

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論　文　内　容　の　要　旨

Supercritical fluid extraction (SFE) is recently considered as a novel technology due to fast, efficient, and clean method. It has been used to recover the bioactive compounds from plant materials in food, pharmaceutical, and cosmetic industries because it is able to generate the high purity of product without the degradation of the active compounds and the toxic residues. In compare to conventional methods, SFE has some advantages such as higher diffusion coefficient, lower viscosity and surface tension than the liquid solvent. The selectivity of supercritical fluid is better than that of conventional solvent because its solvation power can be adjusted by changing temperature and pressure. The most used solvent in SFE is carbon dioxide. Carbon dioxide is known as a safe, nontoxic and cheap solvent. Carbon dioxide has low polarity, which makes it easily to extract lipids, fats, and non-polar substances. However, some bioactive compounds from plant materials are difficult to extract by using carbon dioxide due to the high polarity of bioactive compounds. Therefore, the small amount of polar solvents (modifiers) is frequently added in order to increase the dissolving power. Generally, the parameters that may affect the extraction efficiency in SFE include temperature, pressure, solvent flow rate, extraction time, and among others. The extraction efficiency is also influenced by the morphology of the solid subtract particle because the solvent must go through the diffusive paths inside the solid particle in order to extract the specific compounds. The efficiency of SFE process is able to improve by using combined extraction techniques such as supercritical fluid expended liquid extraction, ultrasound assisted SFE, and SFE integrated hydrothermal process. Supercritical fluid method has not only been used to extract bioactive or valuable compounds from plants, industrial by products, algae and microalgae, and extract the toxic compounds and the metal from solid and liquid environmental matrices, but also used for micronization and particle formation. Therefore, this method recently has been applied in food and natural products extraction, pharmaceutics, and environment. In this thesis, the application of process integration of supercritical fluid with novel techniques in extraction and microencapsulation of bioactive compounds from plant materials was investigated. The thesis consists of four chapters.

In chapter 1, the supercritical fluid technology are briefly reviewed. The theory and the advantages of supercritical fluid technology over conventional methods are also concisely given.

In chapter 2, the application of process integration of supercritical with novel techniques in extraction of the bioactive compounds from plant materials including garlic husk, turmeric, Khmer medicinal plants was investigated. The phenolic compounds and antioxidant activity extracted from garlic husk by using carbon dioxide expanded ethanol (CXE) was studied. The extraction was carried out in ranges of temperature (50-200 °C), CO_2 flow rate (0.5-2 mL/min) at constant pressure (10 MPa). Total phenolic compounds and antioxidant activity were successfully extracted using CXE. The high amount of total phenolic compounds of 56.26 mg GAE/g of dried garlic husk and antioxidant activity (IC_{50}) of 0.41 mg/mL was achieved at temperature of 200 °C and CO_2 flow rate of 0.5 mL/min. The five major phenolic compounds identified in CXE extract were garlic acid, 4-hydrobenzoic acid, caffeic acid, p-coumaric acid, and trans-ferulic acid. Compared to the pressurized ethanol, the result showed that CXE not only improved the extraction yield, but also increased the total phenolic compounds and antioxidant activity in extract. Moreover, the amount of total phenolic compounds and antioxidant activity extracted with CXE surpassed those obtained with Soxhlet extraction. Additionally, the extraction of curcumin from turmeric using ultrasound assisted supercritical carbon dioxide (USC-CO_2) was also evaluated. The extraction was performed at 50 °C, 25 MPa, CO_2 flow rate of 3 mL/min with 10% cosolvent. The result of extraction, thermogravimetry (TG), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscope (SEM) showed that ultrasound power could disrupt cell wall and release the target compounds from turmeric. The result also showed that USC-CO_2 could provide higher curcumin content in the extract and faster extraction time compared to SC-CO_2 extraction without ultrasound. Furthermore, the comparison of conventional and USC-CO_2 extraction of curcumin from turmeric was carried out in this chapter. The effect of operating conditions on extraction including temperature (40-60 °C), pressure (15-25 MPa), extraction time (30-120 min), CO_2 flow rate (2-4 mL/min) and percentage of cosolvent (10-20% v/v) were also studied. The result showed that the high extraction yield of 7.17% w/w and curcumin content of 1.69% w/w were achieved at temperature of 50 °C, pressure of 25 MPa, extraction time of 90 min, CO_2 flow rate of 3 mL/min with 10% cosolvent. Compared to conventional method, USC-CO_2 could provide higher curcumin content in extraction yield in a shorter extraction time. SEM, TG, and FTIR was used to analyze turmeric undergoing USC-CO_2 and conventional extraction and showed that ultrasound could break down the cell walls and remove some functional groups from plant materials, resulting an increase the selectivity of compounds in extraction yield. In addition, extraction of phytochemical constituents in Khmer medicinal plants (*Dialium cochinchinense* Pierre, *Cinnamomum cambodianum* Lecomte, *Gardenia angkorensis* Pitard, *Dialium cochinchinense* Pierre, *Cananga latifolia* (Hook. f. & Thomson) Finet & Gagnep, and *Oroxylum indicum* (L.) Kurz bark) using supercritical carbon dioxide integrated hydrothermal process (SC-CO_2-H) was studied in this chapter. The extraction was performed at temperature of 150 °C, pressure of 10 MPa, water flow rate of 2 mL/min, CO_2 flow rate of 0.15 mL/min, and extraction time of 4 h. The total phenolic compounds and antioxidant activities of Khmer medicinal plants were determined by using Folin Ciocalteu and DPPH scavenging activity method. The phytochemical constituents in Khmer medicinal plants were identified using GC-MS. The results suggested that the extract of Khmer medicinal plants obtained by SC-CO_2-H contained the high amount of total phenolic compounds with potential antioxidant activity. Compared to conventional methods, the total phenolic compounds content in SC-CO_2-H extracts were higher than that in Soxhlet methanol and hot water extract. The antioxidant activity in SC-CO_2-H extract was higher than that of hot water extract, but it was a little bit lower compared with that of Soxhlet methanol extract. The result of GC-MS suggested that Khmer medicinal plants had many bioactive compounds with a board range of biological activities that could be applied in food, pharmaceutical, and cosmetic industries.

The application of supercritical carbon dioxide as an anti-solvent for micronization of curcumin with biodegradable polymer was determined in chapter 3. The effect of swirl mixer operating parameters such as curcumin/PVP ratio, feed concentration, temperature, pressure, and CO_2 flow rate was investigated. The characterization and solubility of particles were determined by using SEM, FTIR, and UV spectrophotometry. The result showed that spherical particles of curcumin/PVP were successfully fabricated by supercritical carbon dioxide (anti-solvent) using micro swirl mixer. The best condition for production of curcumin/PVP particles by supercritical anti-solvent (SAS) process with swirl mixer was at curcumin/PVP ratio of 1:30, feed concentration of 5 mg/mL, temperature of 40 °C, pressure of 15 MPa, and CO_2 flow rate of 15 mL/min. In addition, the dissolution test showed that the dissolution of curcumin/PVP particles was faster than that of raw curcumin.

In this study, the bioactive compounds from different plant materials, such as garlic husk, turmeric, and Khmer medicinal plants, were successfully extracted by using the process integration of supercritical fluid with novel techniques including CXE, USC-CO_2, and SC-CO_2-H. Compared to conventional methods, the process integration of supercritical fluid with novel techniques not only could increase the selectivity of compounds in extraction yield, but also reduced the extraction time. Furthermore, SC-CO_2 with micro swirl mixer also showed potential ability in microencapsulation of curcumin in PVP. Spherical curcumin/PVP particles obtained by this method could improve the dissolution rate of curcumin. Based on these results, it can be concluded that the process integration of supercritical fluid with novel techniques is an efficient method in extraction and encapsulation of bioactive compounds. The process integration of supercritical fluid with novel techniques is also possible to apply in the industrial application. However, the special effort should be acquired to solve the theoretical and technical problems such as thermodynamic constraints of solubility and selectivity, kinetic constraints of mass transfer rate, modeling of combined extraction methods for better understanding of extraction mechanism and optimization.