Research Activity on Supercritical Fluids Technology at Kumamoto University

Supercritical CO_2 Extraction/Fractionation and Hydrothermal Extraction of Valuable Compounds from Natural Products

As mention above, CO_2 is the best supercritical fluid in the field of extraction of heat-sensitive components mainly destined for human nutrition, due to its chemical and physical properties and its low critical temperature and pressure values. Recently, water has also been used as supercritical solvent for extraction, especially in the field of food application. Water is polar component that environmentally friendly solvent, especially for non heat-sensitive compounds.

Extracts obtained by means of supercritical fluid extraction are of higher quality than those obtained by organic solvent extraction or by water and steam distillation, which can induce thermal degradation, hydrolysis and water solubilization of some components. In short, supercritical CO_2 extraction offers high selectivity in the extraction compounds, shorter extraction period; no solvent residue, with positive effects on extract quality. Also, the flexibility in the control of the variables involved in the supercritical fluids extraction process allows one to optimize the experimental conditions, considering the manipulation of a substance of interest.

In our laboratory, valuable compounds including essential oil, carotenoids, fatty acids, phenolic compounds and alkaloids have been extracted from various natural products using supercritical CO_2 and water at hydrothermal condition. Schematic diagram of supercritical CO_2 and hydrothermal extraction apparatus are shown in Figure 1 and 2, respectively. The apparatuses are mainly consisted of high pressure pumps, extractor with the heater, back pressure regulator, extract collector, chiller and gas meter for CO_2.

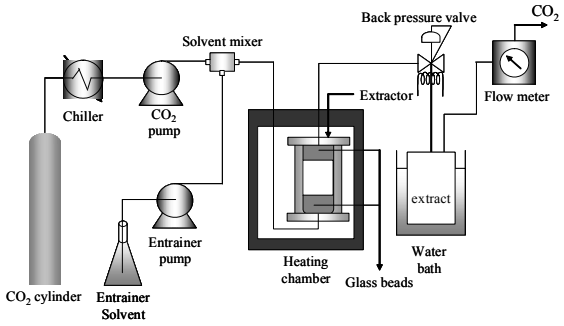

Figure 1. Schematic diagram of supercritical CO_2 extraction apparatus.

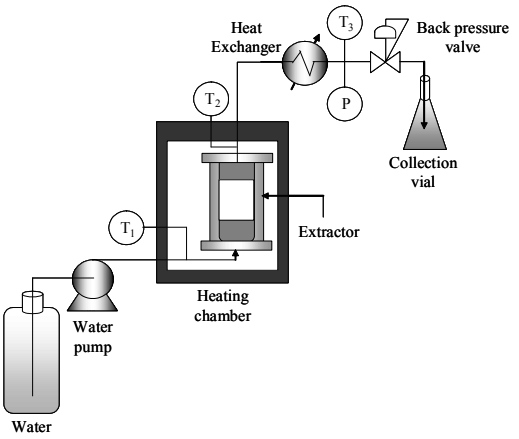

Figure 2. Schematic diagram of hydrothermal extraction apparatus.

Essential oils are mainly formed by hydrocarbon and oxygenated terpenes and by hydrocarbon and oxygenated sesquiterpenes. Usually, they are used as flavor in food and beverage. From the point of view of supercritical fluids extraction, essential oil isolation is an example of extraction plus fractional separation. Indeed, this process can be optimally performed operating at mild pressures (from 9 to 20 MPa) and temperatures (from 40 to 50^{o}C) since at these process conditions all the essential oil

component are largely soluble in SC-CO_2. Essential oil from sunflower seeds [2], geranium [3], nutmeg seeds [4], Turkish plants [5] and Japanese pepper [6] have been extracted with supercritical CO_2. Composition and yield of extracted essential oils were analyzed qualitatively and quantitatively using GC-MS and GC-FID, respectively. Optimum extraction condition of each material was also reported. We also developed fractionation of essential oils (bergamot [7-8], citrus oil [9], lemon oil [10] and yuzu oil [11]) using supercritical CO_2 to selectively separate a valuable compound. Moreover, fractionation of α-tocopherol from oil using supercritical CO_2 was also investigated [12].

Vegetable oils are a natural, convenient source of such unsaturated fats. Vegetable oil is usually produced from ground seeds. We have presented supercritical CO_2 extraction of oil from seeds [13-15]. Seed oils mainly formed by triglycerides are readily soluble in supercritical CO_2 at 40^{o}C and at pressure larger than 20 MPa. The main parameters for this process are particle size, pressure and extraction time. Small particles and high pressures can increase extraction rate in order to reduce extraction time. In some cases, after extraction process, the supercritical CO_2 triglycerides solution is separated in the separator at sub-critical conditions to reduce to near zero the CO_2 solvent power and allow the recovery of oil. Furthermore, oil free gaseous CO_2 is obtained in the separator.

Carotenoids are the most famous one of the nutraceuticals. Carotenoids are a family of compounds that possess antioxidant and colouring properties for food, cosmetic and medical applications. They can be obtained in a large variety of natural sources, such as vegetables, animals, yeasts and microalgae. Carotenoids have very low or moderate solubilities in supercritical CO_2. Therefore, in several cases supercritical CO_2 has been added with a co-solvent. The most frequently used co-solvent is ethanol since its presence in the extracts does not compromise the use in food applications. However, it is better, when it is possible, to avoid the use of a co-solvent that tends to regenerate the problem of solvent elimination from the extracts. In our group, carotenoids, such as β-carotene, lycopene, astaxanthin and lutein, have been extracted from tomato pulp [16], microalgae [17-20] and rosehip [21], using supercritical CO_2 with or without co-solvent. Carotenoids have been successfully extracted with high yield and selectivity. Optimization of process, extraction rate and solubility of carotenoids in supercritical CO_2 were reported. Generally, carotenoids yield strongly increased with increasing temperature due to their localization in the plants matrix.

Polyphenolic compounds are containing a polyphenolic substructure that has medicinal properties. The main source of polyphenol is nutritional, since they are found in a wide array of phytonutrient-bearing foods, such as fruits and vegetables. Polyphenolic compounds are separated into two categories flavonoids and non-flavonoids. Flavonoids include anthocyanins and tannins which contribute to the color and mouthfeel of fruits and vegetables, which have violet color. Non-flavonoids include stilbenes such as resveratrol and compounds derived from acids in fruits and vegetables like benzoic, caffeic and cinnamic acid. In grapes, phenolics are found widely in the skin, stems and seeds. They are water soluble and will often secrete into the vacuole of grape berries as glycosides. However, they have very low solubilities in supercritical CO_2. Furthermore, addition of co-solvent with high polarity is necessary to enhance CO_2 power. We have investigated polyphenolic compounds having antioxidant activity extraction from various plants using supercritical CO_2 with co-solvent and water at hydrothermal condition [22-25]. Moreover, other extraction methods were also

developed to obtain higher extraction yield of polyphenolic compounds [26-27]. Except polyphenolic compounds, extraction of other biological active compounds from various plants, such as Turmeric [28], biwa seeds [29], rice bran [30], citrus peel [31] and mushroom [32], were also investigated.

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