

Extraction of Bioactive Macromolecules Using Supercritical Carbon Dioxide Technology

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Abstract. The complex structure of biological macromolecules makes it play an important role in various fields, and its demand is increasing day by day, so its extraction technology has also become a hot research object. For example, essential oils can be able to extract from different parts of plants. Because they are rich in a variety of special chemical groups, they endow them with unique physical and chemical properties, making them widely used in various fields. Conventional extraction methods, such as steam distillation, cold pressing and solvent extraction, all have certain limitations and disadvantages for extracting essential oils. And with the intensification of the greenhouse effect, how to use carbon dioxide (CO₂) has become a hot and urgent topic. The supercritical CO₂ extraction technology is to turn CO₂ into treasure and use it rationally. The use of supercritical CO₂ extraction has higher efficiency, lower energy consumption and other outstanding advantages. This research focuses on the analysis of the extraction rate of biologically active macromolecules by supercritical CO₂ extraction technology, including essential oils and Angelica. The effects of different experimental parameters on the extraction results were explored, the reasons were analyzed, and appropriate extraction conditions were obtained to optimize the extraction rate.

Keywords: Supercritical CO₂, Extraction, Macromolecule.

1. Introduction

Biomacromolecules are important components of living organisms, with large molecular weights and complex structures. The complex structure endows special properties, so biomacromolecules have the advantages of high biocompatibility, biometabolism, low immunogenicity, and high targeting. In recent years, biomacromolecules show an extremely key role in major and rare diseases, so the demand for biomacromolecules has gradually increased. Different extraction methods have been widely used to extract these functional biomacromolecules. However, these extraction methods still have some disadvantages. As a result, optimizing the extraction of biomacromolecules has also become a work of great research significance.

Supercritical fluid extraction technology is a green and environmentally friendly emerging hot technology that utilizes fluid to separate at supercritical temperature and supercritical pressure. In the supercritical state, carbon dioxide (CO₂) has the following characteristics. It will happen when pressure and temperature change, has a high density and dielectric constant and a potent capacity to dissolve the majority of compounds, and separates much more quickly than liquid extraction. As a result, the supercritical CO₂ extraction methods not only allows for the selective solubility of certain chemicals but also makes it simple to separate the solvent from the extract. Supercritical CO₂ extraction technology provides benefits over traditional separation techniques, including low extraction temperature, excellent selectivity, high efficiency, low energy consumption, and no pollution [1]. As a result, this technique is ideal for use in a variety of industries, including biopharmaceuticals, the food sector, refined petroleum, health care goods, cosmetics and perfumes. This research will analyze and discuss the performance of supercritical CO₂ in the extraction of Angelica and essential oils. Different influencing factors such as experimental temperature and

sample size were analyzed when the supercritical CO₂ extraction method is used to extract different biologically active macromolecules.

2. Extraction of Angelica

Angelica sinensis is a multispecies of Umbelliferae, about a hundred species. It is also a widely used Chinese herbal medicine. *Angelica sinensis* can not only treat some gynecological diseases, such as dysmenorrhea, irregular menstruation and so forth, but also in some modern pharmacological research. For example, *Angelica* appears to have a certain protective effect on vascular endothelial cells damaged by oxidative stress [2]. And the extract of *Angelica sinensis* has a certain improvement effect on liver and kidney damage in aging mice. This also means that *Angelica* and its extracts are of great significance to the development of the medical field in China and even the world. The main effective bioactive molecules of *Angelica* are kaempferol, ferulic acid, ligustilide and Z-butylene phthalide and so on. For this reason, the extraction of bioactive macromolecules with multifunctional uses from *Angelica sinensis* has received more and more attention.

At present, the extraction methods for effective biologically active molecules of *Angelica sinensis* are mainly supercritical CO₂ technology and molecular distillation technology. Because both of these two separation methods use vacuum technology to minimize the temperature during the separation process, so as to prevent the target active molecules from being decomposed. In both extraction methods, molecular distillation technology will face problems such as poor oil-water separation, while supercritical CO₂ shows the disadvantage of less than ideal extraction effect for polar substances. To this end, here, this part will mainly analyze the application performance of supercritical CO₂ in the extraction of *Angelica sinensis* and its biologically active macromolecules. In supercritical CO₂ technology, the effect of different experimental conditions on substance extraction will be further discussed, including system pressure, CO₂ flow rate, and system temperature.

2.1. Analysis of different influencing factors

For the temperature of the system, raising the temperature of the system will improve the extraction efficiency. But at the same time, the state of the supercritical fluid will change, and the density will decrease, resulting in the extraction effect is not ideal. For example, in the oil extraction project of babassu seeds, the temperature condition of supercritical CO₂ extraction was increased from 60 °C to 80 °C, and the output oil yield was increased from 86.56% to 89.86%, Appropriately increasing the temperature is beneficial for the extraction to proceed fully [3].

For the system pressure, it should be selected based on experimental conditions and extraction economy. Appropriately increasing the pressure will certainly improve the extraction efficiency, but it will also increase the requirements for the equipment. Therefore, selecting the appropriate system pressure is an indispensable part of the extraction step. In the pharmaceutical production industry, constant temperature and high pressure to adjust the density of the solvent is dominant, and the solvent concentration used has a significant effect on the extraction efficiency of the target substance, which generally shows a positive correlation [4]. For example, under this condition, the extraction rate increases significantly.

For CO₂ flow, by increasing the CO₂ flow within an appropriate range, the distance between the operating line and the balance line can be increased, the mass transfer driving force will also increase, and the extraction efficiency will also increase [5]. However, an excessive flow rate will also lead to a decrease in the extraction efficiency, which was also confirmed by Johner et al. [6]. This is because the flow and flow rate of CO₂ increases under turbulent flow, improving the relationship between the solute components and the solvent in the natural product matrix. This also reduces the external mass transfer resistance, resulting in a more ideal extraction rate [7]. However, when the flow rate or flow exceeds the optimal value, it will lead to unsuccessful experimental extraction in some experiments. For example, in the extraction experiment of the seed oil of milk thistle, when the flow rate of CO₂ was greater than 4 mL/min, a large amount of the resultant product was not obtained [8].

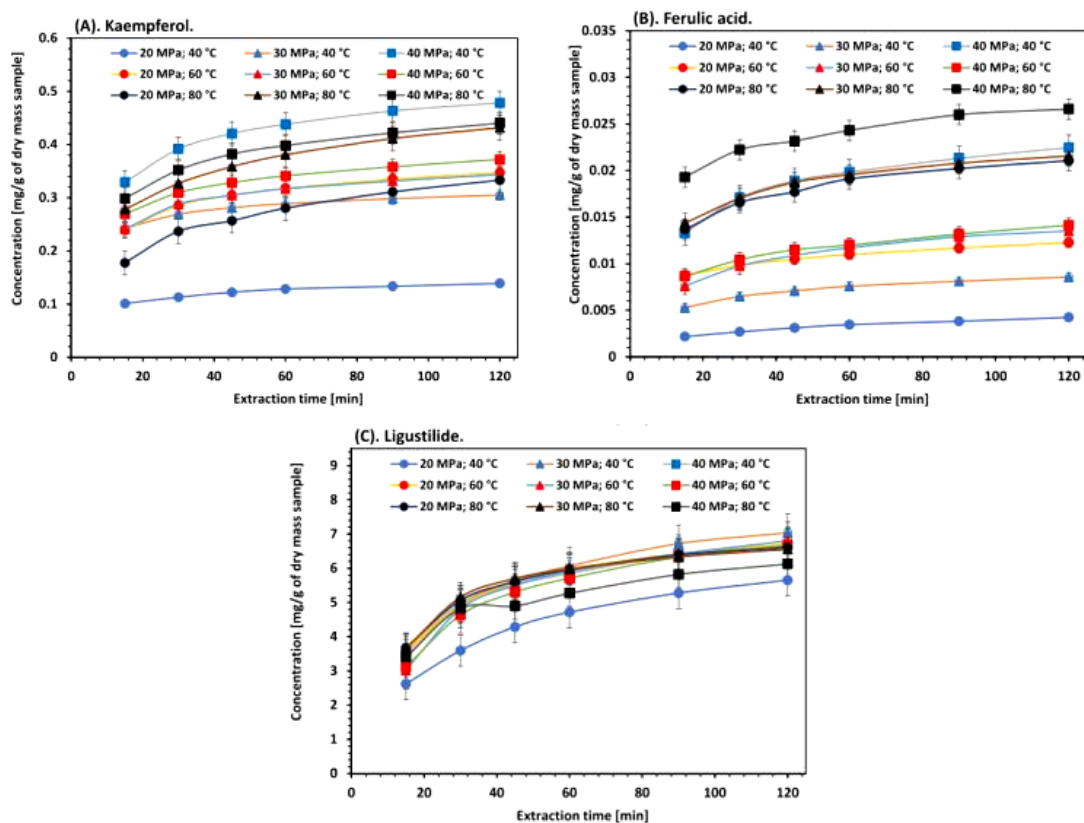


Figure 1. Concentration of kaempferol, ferulic acid and ligustilide [9]

2.2. Comparative analysis of different extraction methods

The polarity of CO₂ is relatively low. When the supercritical CO₂ extraction technology is used to extract polar bioactive molecules, the extraction efficiency will be low. For example, when the supercritical CO₂ method is used to extract *Angelica* [9], a certain amount of ethanol co-solvent was added to it to improve the solubilization ability of CO₂. The product content comparison is shown in Figure 1, which are kaempferol, ferulic acid, and ligustilide. It can more clearly see the effect of temperature and pressure on supercritical CO₂ technology when extracting biological macromolecules.

Table 1. The ligustilide extracted from *Angelica sinensis* [10].

Serial Number	Weig(kg)	Mixed volatile oil (ml)	Ligustilide amount (g)
1	10	351.0	53.54
2	10	355.4	54.21
3	10	358.7	53.25
Average	10	355.0	53.67

In the molecular distillation method, some people also use steam distillation-menthol extraction method to extract ligustilide in *Angelica* [10]. Using this method to extract *Angelica sinensis* can also effectively extract ligustilide in *Angelica sinensis*. The average ligustilide concentration in the Table 1 is converted into units to obtain about 5.3 mg of ligustilide per gram of *Angelica sinensis* by steam distillation-menthol extraction. It can be obtained that under the conditions of appropriate temperature and extraction time, the content of ligustilide per gram of *Angelica sinensis* extracted by supercritical CO₂ extraction technology is significantly higher than that of steam distillation-menthol extraction method. Even under the conditions of 30 MPa at 40°C, about 7mg of ligustilide per gram of *Angelica sinensis* was extracted.

From the data point of view, in contrast, supercritical CO₂ extraction efficiency has a great advantage. Wu et al. [11] used the temperature-controlled hydrophobic ionic liquid ultrasonic/heating-assisted extraction method to obtain ferulic acid. First, under the conditions of ultrasonication and heating,

the hydrophobic ions are dispersed into the solution, and then centrifuged at 0 °C. At 60°C, the average extraction content of ferulic acid was about 0.83 mg/g. In contrast, the supercritical fluid extraction method was used to extract the ferulic acid in *Angelica sinensis*. At 60 °C, the extraction content of ferulic acid was 0.014 mg/g. This also shows that in the extraction results of ferulic acid, supercritical CO₂ extraction technology is not ideal for temperature-controlled hydrophobic ionic liquid ultrasonic/heat-assisted extraction. Compared with the ultrasonic/heat-assisted extraction of temperature-controlled hydrophobic ionic liquid, the effect of supercritical CO₂ extraction of ferulic acid is not ideal, it shows that there is still room for improvement in supercritical CO₂ extraction technology, which also shows the strong development potential of supercritical CO₂ extraction technology. However, considering various factors, supercritical CO₂ extraction technology not only has the advantages of safety and no other solvent residues as compared to other molecular distillation methods, but also the extraction efficiency of lipid components of some natural products is also much higher than that of traditional extraction processes.

3. Extraction of essential oils

It can extract essential oils from plants such as roots, stems and leaves, and these oils are sometimes called aromatic oils or volatile oils. Hydrocarbons and compounds containing oxygen, such as phenols, ethers, acids, esters and alcohols, make up the majority of its chemical makeup. Because of the existence of these special chemical groups, essential oils have unique physicochemical properties such as long-lasting fragrance, antibacterial and antioxidant activities. Therefore, essential oils are widely used in cosmetics, fragrances, food and pharmaceutical fields. Many extraction methods, such as microwave extraction and solvent extraction, can be widely used for the extraction of a diverse of various essential oils. However, there are drawbacks to the conventional extraction procedure, including limited yield and subpar product. Compared with traditional extraction methods, supercritical CO₂ may increase essential oil production, use less energy, prevent heat-sensitive components from decomposing, and maintain the quality of essential oils [12]. As a result, supercritical CO₂ have been widely used to extract different essential oils. This section will describe the supercritical CO₂ extraction of several common essential oils.

3.1. Oil extraction from the skin of a tangerine

Citrus peel is converted into tangerine peel essential oil. It has physiological activities such as antioxidant, anti-cancer, and antibacterial. It is widely used in the treatment of indigestion, cough, chest tightness and other diseases. In addition, due of its strong scent, the essential oil from tangerine peel is often added to cuisine as a spice to improve taste. The operation steps of using supercritical CO₂ extraction are as follows. The tangerine peel sample is loaded into the extraction container, and the CO₂ in the gas cylinder is sent to the CO₂ container, where it is transformed to supercritical CO₂ before being pumped through the whole extraction apparatus. After gathering pertinent operational factors including pressure and extraction time, the yield was calculated using the amount of tangerine peel essential oil that had been collected and weighed. The extraction yield is impacted in different ways by various process factors, such as extraction time, temperature, pressure and particle size.

To analyze the effect of tangerine peel particle size on the extraction rate, the extraction rate of tangerine peel with five particle sizes (0.2-1.0 mm) was tested. As shown in Figure 1a, the results show that the yield of dried tangerine peel oil is increased significantly as the decreasing in particle size [12]. The used sample with smaller particle size can increase the contact area between the supercritical CO₂ fluid and the tangerine peel powder. However, beyond a certain point, the extract is quickly reabsorbed by the tangerine peel's surface, which lowers the pace at which it diffuses. It was investigated how temperature between 35 and 55 °C affected the rate of tangerine peel essential oil extraction. As shown in Figure 1b, the results show that the yield of tangerine peel essential oil rises with temperature between 35 and 45 °C, but it rapidly falls beyond that point and reaches a maximum yield of 0.89% at 45 °C [12]. The primary reason is that as temperature rises, the extract's diffusion rate accelerates,

increasing the production of tangerine peel oil. However, as temperature rises, supercritical carbon dioxide fluid's density decreases, decreasing the yield of essential oil.

The used pressure in the experiment is another important factor in the extraction of essential oils with supercritical CO₂. As shown in Figure 1c, the results shown that the output of the essential oil is significantly influenced by pressure. From the outset, the yield of essential oil had a rising trend. It swiftly increased to its highest value of 1.11% at 15 MPa. The curve begins to flatten as the pressure exceeds 15 MPa. The output hardly changes when the pressure is increased from 25 to 30 MPa [12]. The effect of pressure is due to the fact that the specific mass of supercritical CO₂ increases with increasing pressure, thereby enhancing the solvation capacity of supercritical CO₂. However, when the pressure is too high, the rate of supercritical CO₂ mass transfer and diffusion will stop the yield of essential oil from increasing. Additionally, greater pressures may be used to produce tangerine peel oils and waxes, which also degrades the quality of essential oils.

The effect of extraction time on the rate of tangerine peel oil extraction was further investigated. As shown in Figure 1d, the results show that the yield of essential oils rose steadily from 0 to 1.25% while the extraction period was between 0 and 180 minutes, but that the yield hardly increased as the extraction time was extended further. As the extraction time increase, the extraction rate of essential oil continued to decrease. Therefore, in the following RSM surveys, the extraction time was chosen between 90 and 150 minutes in order to more effectively reduce the operation time [12].

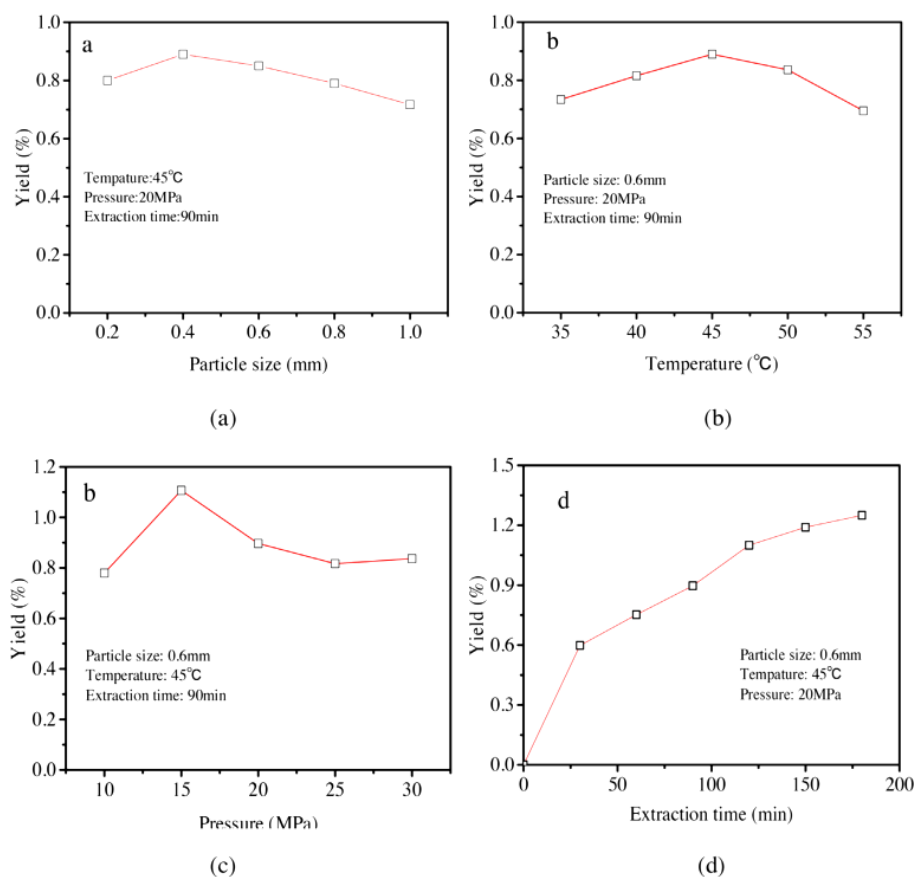


Figure 2. Effects of different experimental conditions on the extraction of essential oils [12]

3.2. Egusi essential oil

Egusi seed is a family of the cucurbit subfamily Benincaseae, which is adapted to summer growing conditions, especially in Africa. Egusi are highly adaptable and able to survive extreme conditions. This Egusi seed is rich in oils. To this end, different extraction methods have been used to extract seed oils. Here, this part mainly introduces the application and performance of supercritical CO₂ technology in the extraction of seed oil.

Temperature has an impact on extraction rate during supercritical extraction at constant pressure in two ways: (1) as process temperature rises, solute vapor pressure rises, increasing solubility; and (2) as solvent density falls, causing solubility to fall. Egusi oil is more soluble and diffusive under high pressures and temperatures, which lowers the barrier to mass transfer. The oil production of Egusi considerably increased at 75 °C and 600 bar (EO3) due to the high temperature and pressure [13]. The reasons for the lower water content of the three oil samples may be related to the lower water content of crude seed oil (5-8% w/w) and the use of high-purity supercritical CO₂. The EO1's protein content was substantially lower than that of EO2 and EO3's. The absence of nutrients from the residue leaking into the oil is what causes the low protein content. This is one of the supercritical extraction process's green processing impacts because the residue it leaves behind after extraction keeps its nutrients and may be converted into useful substances. As a result of the solid matrix separating due to high pressure and temperature, more solid liquid components are released during the extraction process [13]. Myristic acid, undecanoic acid, palmitic acid, and stearic acid make up the fatty acid profile of Egusi. The amounts of undecanoate fatty acids in EO1, EO2, and EO3 were 7.8, 8.0, and 8.3 mg/100 g, respectively, with no appreciable variation between them. In addition to helping in ringworm therapy, undecanoate fatty acid promotes wound healing [13].

4. Conclusions

This research discussed discusses the effect of different experimental conditions including experimental pressure and temperature on the bioactive macromolecules extraction by using supercritical CO₂ technology. At the same time, the molecular distillation method is compared with supercritical CO₂ extraction in this research. And it is obtained that the supercritical CO₂ extraction technology not only has the advantages of safer and no residue, but also has a much higher extraction rate than the traditional process. For the extraction of ferulic acid, the temperature-controlled hydrophobic ionic liquid ultrasonic/heat-assisted extraction process is better than the supercritical CO₂ extraction technology, indicating that the technology still has a certain room for development. For the extraction of essential oils, in addition, this research also analyzed the effect of other experimental conditions on the extraction efficiency of bioactive macromolecules, including particle size and extraction time.

As a new method for the extraction of biologically active macromolecules, supercritical CO₂ technology has shown great advantages in the extraction process. It has the advantages of mild use conditions, ideal permeability, good selectivity, and good safety while having high extraction efficiency. As a result, supercritical CO₂ has broad application prospects, where this technology can exert its unique advantages in various fields such as agriculture, chemical industry, biochemistry and so on. However, there are still many areas for improvement and adjustment in this technology, such as the high cost of equipment, different extraction environments for different raw materials and the separation of entainers, all of which are waiting for us to solve. Better use of supercritical CO₂ extraction technology can not only effectively improve the efficiency of the chemical industry, but also effectively recycle the greenhouse gas CO₂ and reduce its proportion in the atmosphere in the face of global warming. In the near future, through our continuous improvement, supercritical CO₂ extraction technology will be widely used.

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