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Optimization Analysis of Continuous Supercritical Extraction System for *Ganoderma lucidum*

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The Coronavirus disease 2019 (COVID-19) pandemic, which emerged in 2019, has resulted in the continuous emergence of various viral mutants, significantly impacting human life. Concurrently, the importance of preventive healthcare has been increasingly recognized. Existing research has demonstrated that polysaccharides derived from *Ganoderma lucidum* can effectively inhibit the novel coronavirus in animal studies. However, effective inhibition requires specific extraction methods and optimal dosages. To address this challenge, we developed a continuous supercritical extraction (CSE) system designed to purify high-content active ingredients at low temperatures, preserving their biological activity. This system has been successfully applied in trial mass production with low power consumption. Utilizing the Taguchi method for experimental design, we optimized the extraction parameters, thereby reducing the costs associated with verifying the active ingredients in *G. lucidum*. The results of our study identified optimal conditions for polysaccharide extraction, which were compared against various experimental parameter combinations. The findings confirm that the CSE system is not only efficient but also suitable for the successful extraction of polysaccharides, laying a solid foundation for future applications in the industrial processing of natural products.

1. Introduction

Nowadays, the world is still under the shadow of the post-COVID-19 epidemic, and the world hopes for vaccines and drugs to be used. For this reason, in the latest research on the development of Chinese herbal medicine, it is found that extracts of *Ganoderma lucidum* can effectively fight the new coronavirus in animal tests. Relevant results were published in the Proceedings of the National Academy of Sciences of the United States of America (PNAS) in 2021.(1) In view of this, many biotechnology companies have begun to develop functional health foods related to Chinese herbal medicine extraction. Today's extraction technology applications in mass

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production development still mainly rely on traditional purification and separation methods, which are divided into two categories: distillation and solvent extraction. Steam distillation is the most commonly used extraction method. The principle is to use high-temperature water vapor to extract the volatile components in a plant, and then use cooling to precipitate the active ingredients. Finally, the extract can be obtained through the separation process.(2)

The solvent extraction method uses volatile organic solvents and continuous reflux extraction, the solvent is removed from the extract by distillation or vacuum distillation, and a crude product can be obtained. This method has a high acquisition rate, but because the wax components and resins in the plant are also extracted at the same time, resulting in many impurities, further refining and purification steps are needed. The relevant equipment required by this method is also simple and easy to operate. However, although the above two methods have their advantages, they consume large amounts of energy and generate risks of organic solvent residues, which are not conducive to the mass production of biotech products.

Therefore, in this study, we mainly designed a new high-efficiency and energy-saving purification and separation trial production equipment, which can be suitable for the purification and separation of a variety of natural products, and can avoid the use of solvents that are harmful to the human body, reduce extraction energy consumption, and avoid excessive extraction temperatures. This results in advantages such as reduced extraction activity, thereby reducing manufacturing costs and making it easier to carry out subsequent mass production using the equipment.

Owing to the complex composition of natural Chinese herbal medicines, different extraction methods should be used to separate the required ingredients. Currently, there are many different extraction methods, equipment, and processes used. In addition to the above-mentioned distillation and solvent extraction methods, there are also enzyme extraction methods. On the basis of the composition of the plant cell wall, the enzyme reaction is highly specific, and the corresponding enzyme is selected to hydrolyze or degrade the cell wall components to destroy the cell wall structure, dissolve the intracellular components in the solvent, and then achieve the extraction effect.(3) There is also a microwave extraction method(4) that uses microwave radiation to cause substances in plant cells to absorb wave energy and generate heat, which increases the pressure inside the cells, causing the cells to rupture and allowing the target products to flow out. This method requires direct contact with the sample matrix through the solvent to separate the required active ingredients from the sample. However, for some heat-sensitive natural products, very high temperatures will affect the extraction and purification of active ingredients. Moreover, some scientists currently even believe that microwaves will change the molecular structure of the substance itself. Whether it will have an impact on consumers' health in the future is still a question. There is also the Soxhlet extraction method,(5) which is not suitable for industrial mass production owing to its long extraction time, energy consumption, and difficulty in large-scale equipment. In recent years, supercritical fluid (SF) extraction,(6,7) ultrasonic extraction,(8) accelerated solvent extraction,(9) and solid-phase microextraction(10) have also been used. However, the world is continuously paying attention to the issue of energy saving and carbon reduction, so it is hoped to reduce energy consumption and improve extraction efficiency through a continuous supercritical extraction system (CSE) shown in this paper, and provide enterprises with an optimal strategy.

From the above, various extraction technologies have their own shortcomings, high costs, and complex equipment. Therefore, a CSE method is designed and proposed to overcome the above problems. It can be used as the main purification method for research and industrial mass production.

2. CSE System

In this study, we mainly established a novel CSE system and production process, and we used *G. lucidum* that has antiviral effects as an experimental planning and verification platform for extracts. Most natural products or Chinese herbal medicines have components with different polarities, and polarity is absolutely related to solubility. Therefore, this key factor must be carefully considered when developing extraction processes. As early as 1964, some scholars proposed using solubility parameters to describe the dissolution phenomenon between substances, and its definition is shown in Eq. (1).[11]

$$\delta = \left(\frac{E_{vo}}{V_{mol}} \right) \tag{1}$$

Here, δ represents the dissolution parameter of the substance [$(\mathrm{cal/cm^3})^{0.5}$], E_{vo} is the evaporation energy of the substance when the pressure is zero, and V_{mol} is molar volume. If the solubility parameters of the solvent and solute are similar, they will dissolve in each other. Therefore, the polarity of molecules has a significant impact on the solubility of substances. Polar molecules are easily soluble in polar solvents, whereas nonpolar molecules are easily soluble in nonpolar solvents, known as "like dissolves like". Polar molecules such as sucrose, ammonia, and ionic compounds like sodium chloride are soluble in water. Nonpolar organic compounds with long carbon chains, such as the components of fats, are often insoluble in water but soluble in nonpolar organic solvents. Currently, there are many methods available for extracting polar substances, but the extraction of low-polarity and nonpolar substances remains an area for further development, and is the main focus of this study.

When the fluid is in a supercritical state, the physical properties of the fluid are between those of gas and liquid phases. For example, its viscosity is closer to that of a gas and its density is similar to that of a liquid. Since the density of SF is high and the viscosity is low, when this fluid transports substances, the power required is lower than that of liquids.[12] The diffusion coefficient of SF is more than 10 to 100 times that of a liquid, and there is almost no surface tension. Therefore, when used in extraction, SF can easily penetrate into the tissue, and the mass transfer is much faster than that of a liquid. In this study, we used carbon dioxide as SF. After carbon dioxide enters the supercritical state, it will become organically friendly. Therefore, the fluid has a very high ability to dissolve organic material, and its density is similar to that of a liquid, making the SF more likely to dissolve into the extract and precipitate the solute, thereby becoming a good nontoxic solvent with an excellent extraction effect. When a solute molecule is in SF, if the attraction between the molecule and the solvent is greater than that between the solvents, the solute molecule will be surrounded by the solvent molecules, which is a swarming effect.[13] This effect is considered to be the main reason for the increase in solubility. The solubility of SF is strongly linked to its density, which in turn is affected by variations in temperature and pressure. As a result, by manipulating the temperature and pressure, it is possible to regulate solubility, allowing for the extraction and separation of different components. This capability sets it apart from other extraction methods and conventional solvent extraction.

When a substance is in a supercritical state, active ingredients with different polarities can be extracted in batches by adjusting the pressure. After each extraction, the pressure is decreased and the substance is cooled to return to room temperature and pressure. This process effectively separates and purifies the extracted substances. SE combines the features of solvent extraction and distillation separation. Supercritical carbon dioxide, which is normally a gas, can avoid solvent residue issues and extract heat-sensitive substances without damaging the active components of natural substances owing to its critical temperature of 31.2 °C.

The proposed CSE system consists of an extraction tank, a high-pressure pump with sensors, a temperature-sensing module, and collection tanks. The system pressurizes carbon dioxide to a supercritical state using a high-pressure pump; then, SF enters the extraction tank where the extraction process temperature is controlled. Next, controlled decompression and cooling are used to precipitate the extracted substances for separation and purification. Figure 1 shows the preliminary system design and pipeline configuration of the expected mass production equipment, whereas Table 1 lists important equipment specifications and related elements.

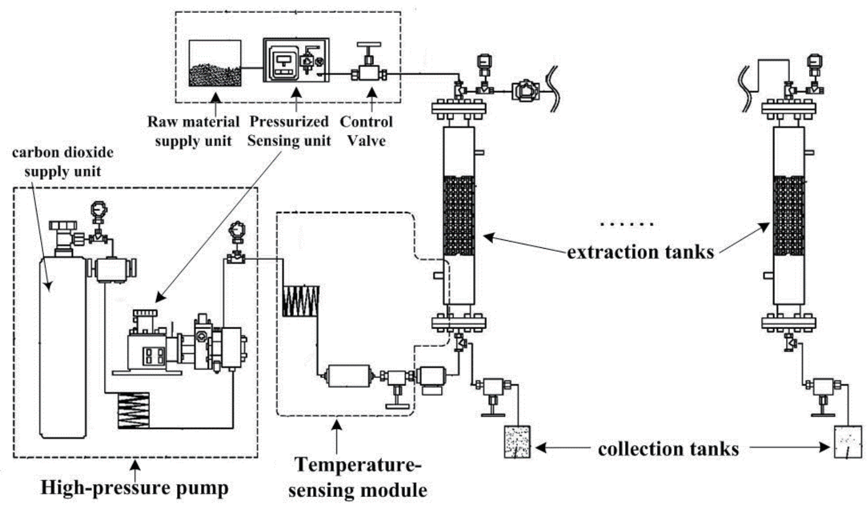

Fig. 1. Design of CSE system.

Table 1
Factors and levels of Taguchi method.

Factors	Levels		
	1	2	3
A. Extracting Pressure (MPa)	16	26	36
B. Supercritical Carbon Dioxide Fluid Temperature (°C)	40	50	60
C. Volume Flow Rate of Extract (ml/s)	1/60	1/30	1/20
D. Volume Ratio of Extract to Ethanol (%)	35	55	75

3. Extraction Experiments

Extraction experiments were mainly conducted on *G. lucidum*, which has been proven to be effective,[3] and by the Taguchi method with the L9 (3^4) orthogonal array supplemented by two replications. The relevant factors with three levels including supercritical carbon dioxide temperature, extraction pressure, volume flow rate of extract, and volume ratio of extract to ethanol are shown in Table 1. The larger the better is considered for the quantity of extracted *G. lucidum*. Each factor has different extraction condition settings based on the different substances to be extracted. After the experiment is completed, the active ingredients contained are analyzed by high-performance liquid chromatography (HPLC), and optimized parameters to improve the extraction efficiency can also be obtained.

The larger the better quality loss was explained with the signal-to-noise (SN) ratio and calculated using Eq. (2).

$$S / N = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \tag{2}$$

Here, y_i indicates responses for experiment results and n is the number of repetitions of the experiment.

4. Results and Discussion

The primary active component of *G. lucidum* is its polysaccharide. Following extraction via the CSE system, HPLC was employed to analyze the polysaccharide content. The optimized extraction parameters were evaluated, and the resultant component content was compared with that obtained from alternative parameter combinations through confirmation experiments. In the planning phase of the experiments, average values, standard deviations, and SN ratios were calculated by organizing and processing the experimental procedures according to the Taguchi method. The analysis of polysaccharide content utilized a “larger is better” characteristic, which facilitates the determination of factor responses and the variance analysis. On the basis of these results, optimal parameter conditions for the extraction process were established. The factors and their respective levels are summarized in Table 2, whereas the experimental outcomes—including extraction quantities, average values, and SN ratios—are presented in Table 3.

Figure 2 illustrates the factor response pertaining to the extraction of *G. lucidum* polysaccharides. The values for A1, B1, C1, and D1 are 4.85, 5.82, 6.39, and 5.3, respectively. In comparison, the values for A2, B2, C2, and D2 are 6.17, 5.91, 5.6, and 5.9, respectively. The values for A3, B3, C3, and D3 are recorded as 5.87, 5.16, 4.9, and 5.69, respectively. Thus, the optimal parameter combination identified is A2B2C1D2, highlighted in red in Fig. 2.

Subsequently, confirmation experiments were conducted using the optimized parameter combination to extract *G. lucidum* polysaccharides. The component contents obtained from other parameter combinations were also compared. The experimental results indicated that the optimal parameters—A2 (control pressure: 26 MPa), B2 (control temperature: 50 ℃), C1

Table 2
Experimental parameters are planned by the Taguchi method with L9 orthogonal array.

Experiment No.	A. Extracting Pressure (MPa)	B. Supercritical Carbon Dioxide Fluid Temperature (°C)	C. Volume Flow Rate of Extract (ml/s)	D. Volume Ratio of Extract to Ethanol (%)
1	16	40	1/60	35
2	16	50	1/30	55
3	16	60	1/20	75
4	26	40	1/30	75
5	26	50	1/20	35
6	26	60	1/60	55
7	36	40	1/20	55
8	36	50	1/60	75
9	36	60	1/30	35

Table 3
Experimental results for polysaccharide content by Taguchi method.

Experiment No.	Quantity of polysaccharide (mg/g)		Average quantity of polysaccharide (mg/g)	SN ratio
	1st exp.	2nd exp.		
1	1.9211	1.834	1.87755	5.465
2	1.9012	1.812	1.8566	5.367
3	1.5458	1.523	1.5344	3.718
4	2.145	2.032	6.68325	6.387
5	1.829	1.894	6.88845	5.393
6	2.031	2.341	6.91205	6.727
7	1.836	1.982	11.1178	5.597
8	2.15	2.324	12.38975	6.973
9	1.791	1.78	12.05105	5.035

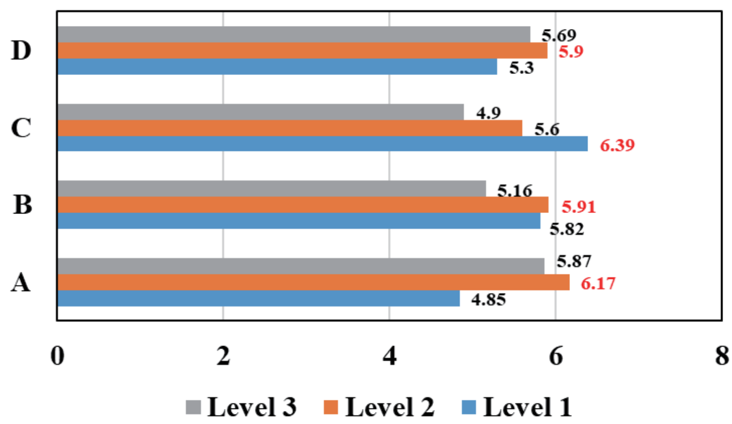

Fig. 2. (Color online) SN ratio of factor and level responses for polysaccharide.

(extract volume flow rate: 1/60 ml/s), and D2 (volume ratio of extract to ethanol: 55%)—yielded a high content of *G. lucidum* polysaccharide at 7.48 mg/g. This confirms that the A2B2C1D2 combination represents the optimized parameters when compared with all experimental data in Table 3.

Table 4
ANOVA of extraction for polysaccharide.

Factor	SS	DOF	Contribution (%)	Confidence (%)
A.	2.87	2	37.04	83.74
B.	1.00	2	12.96	64.30
C.	3.32	2	42.81	85.62
D.	0.56	2	7.19	—

The extraction results obtained from this study demonstrated superior efficacy relative to all results from the Taguchi L9 experiment, thereby validating the accuracy of the optimized parameter combination. Notably, the eighth trial of the L9 design yielded the highest extraction yield among the nine experiments, and when comparing the confirmation experiment results, the extraction rate increased by approximately 7.2%.

Table 4 shows the sum of squares (SS), degrees of freedom (DOF), contributions, and confidence levels associated with each factor. Factor C exhibits the greatest effect on the experiment, accounting for 42.81% of the total variation. Contribution analysis indicates that the volume flow rate of the extract significantly affects the yield of *G. lucidum* polysaccharides. Factor A also impacts the experimental results, contributing 37.04% to the overall variability. These two factors are identified as the primary control conditions for the supercritical extraction of *G. lucidum*. In contrast, the impact of factor D is minimal, accounting for only 7.19%, making it the least contributory factor among all analyzed variables. When combined with the error term, the confidence level for each factor can be assessed. Specifically, the confidence levels for factors A, B, and C are 83.74, 64.30, and 85.62%, respectively. These data suggest a relatively low correlation between factor D and the experimental outcomes.

5. Conclusions

In this study, we successfully developed a novel CSE system, employing *G. lucidum* as a model for validating the extraction process. We elucidated the pivotal role of polarity in the extraction of natural products, demonstrating that solubility parameters are crucial for optimizing extraction methodologies. Utilizing supercritical carbon dioxide (SC-CO_2) as the extraction solvent, we highlighted its efficacy in isolating low-polarity and nonpolar compounds, which pose significant challenges in conventional extraction techniques.

The experimental results revealed that by systematically adjusting extraction pressure, temperature, flow rate, and solvent ratios, we could significantly enhance the yield of polysaccharides from *G. lucidum*. The optimal conditions identified (A2B2C1D2) yielded a remarkable polysaccharide content of 7.48 mg/g, surpassing other parameter combinations and affirming the effectiveness of our optimization strategy. Notably, the eighth trial of the Taguchi L9 design yielded the highest extraction efficiency, with subsequent validation experiments indicating an approximate 7.2% increase in yield.

The CSE system not only improved the extraction efficiency but also mitigated issues associated with solvent residues, making it particularly suitable for the extraction of heat-sensitive compounds. This study contributes valuable insights into the industrial applications of supercritical extraction technologies for natural product processing. The ability to manipulate temperature and pressure to regulate solubility distinctly sets this method apart from traditional extraction techniques, offering a more efficient and environmentally sustainable approach. In conclusion, the findings of this research provide a solid foundation for future investigations in the field of natural product extraction and its industrial applications. Further exploration of the CSE system can lead to advancements in the extraction of bioactive compounds, thereby enhancing the therapeutic potential of natural products across various health applications.

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