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# Research on the Supercritical $CO_2$ Extraction Process of Hetian Rose Essential Oil

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**Abstract:** A longstanding concern in plant essential oil extraction is how to optimize extraction efficiency with limited materials. Supercritical $CO_2$ extraction has been proven effective in enhancing the yield and efficiency of extracting plant essential oils. However, the impact of temperature, pressure, and co-solvent content on extracting Hetian rose essential oil remains unclear. There is a lack of research on the influence of pretreatment methods. This study focuses on investigating supercritical $CO_2$ extraction of rose essential oils from Xinjiang Hetian. The research analyzes the effects of pressure and temperature on the extraction rate and validates the efficiency by calculating the solubility of essential oils in supercritical fluid. Under conditions of 35 MPa, 40 °C, 10 L/h, and a particle size of 0.8 mm, this study evaluates the extraction efficiency using Xinjiang Hetian rose materials pretreated with salt solutions at concentrations of 5%, 10%, and 20%, as well as enzyme solutions at concentrations of 2%, 5%, and 10%. Results indicate that appropriate solution concentration can enhance the extraction effect and mass transfer process, but excessively low or high concentrations do not contribute to improved extraction reactions. The highest extraction rate (8.99%) is achieved using a salt solution concentration of 10%, while the lowest (4.21%) is obtained with a salt solution concentration of 20%.

**Keywords:** supercritical $CO_2$ extraction; rose essential oils; preprocessing method; extraction influencing factors; extraction pressure; extraction temperature

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## 1. Introduction

Xinjiang Hetian roses, cultivated on the edge of the desert, serve as a significant economic crop in Xinjiang. The distinctive regional conditions give rise to variations in oil content between Hetian roses and other rose varieties [1]. The essential oils (EOs) extracted from Hetian roses possess potential broad-spectrum antibacterial and antioxidant properties. They demonstrate remarkable capabilities in terms of both antioxidation and antibacterial effects even at low concentrations, while also offering benefits such as promoting blood circulation and resolving stasis. They are precious concentrated fragrances that serve as crucial raw materials in fragrance manufacturing. There is a high demand for Hetian rose essential oil in the perfume, cosmetics, and medical industries due to its unique scent profile. With the increasing demand for rose essential oils (REOs) within the light industry sector, their applications and societal development continue to expand.

Various methods are utilized for EO extraction, including mechanical pressing, steam distillation, and supercritical $CO_2$ extraction. Mechanical pressing involves physically squeezing plant tissues to extract EOs, which exhibits a lower extraction efficiency and is primarily applicable to citrus fruit peels [2]. Steam distillation utilizes the differing boiling points of components for separation through distillation. This method involves mixing the material with water at a multiple of its volume, heating it for distillation, and then filtering it for separation. While widely applied in extracting EOs from aromatic plants, steam distillation still faces challenges such as low oil quality and extraction efficiency [3].

This makes the optimization of extraction methods for this particular oil of significant commercial value. Supercritical $CO_2$ is a fluid that exhibits characteristics resembling those of a high-density liquid and a low-viscosity gas. It possesses a diffusion coefficient 10 to 100 times higher than that of liquid, resulting in exceptional efficiency in supercritical $CO_2$ extraction [3]. This method yields extracts with remarkable biological activity, effectively addressing the limitations associated with mechanical pressing and steam distillation techniques [4]. It finds extensive application in the extraction of oily substances, making it particularly suitable for REO extraction. Da Porto C et al. [5] conducted experiments on polyphenolic compounds found in roses, confirming the efficacy of supercritical $CO_2$ extraction. The gas-like diffusion and liquid-like solubility properties enable the dissolution of EO components by adjusting the temperature and pressure of supercritical $CO_2$. Subsequently, these compounds can be separated by releasing pressure at the end of the system [3]. In experiments involving lemon EO extraction, Gilani F et al. [6] compared hydrodistillation with supercritical $CO_2$ extraction methods. The results demonstrated superior effectiveness in supercritical $CO_2$ extraction, yielding an extraction rate of 7.6% along with higher content of active ingredients and antioxidant activity when compared to hydrodistillation.

The primary focus of research on the supercritical $CO_2$ extraction of the aforementioned EOs has been to determine optimal extraction conditions for different materials and analyze a limited number of extraction influencing factors. However, there is a lack of experimental studies on EO extraction from Xinjiang Hetian rose materials, despite significant variations in oil content among these materials due to different growth environmental factors within the same plant species. Toluei Z et al. [7] investigated REO content in the Iranian region, ranging from 0.0020% to 0.0190%. In comparison, Najem W et al. [8] determined REO content in Nubari at 0.033–0.065%. Therefore, it is imperative to conduct specific research on supercritical $CO_2$ extraction of EOs from the unique Xinjiang Hetian rose materials in order to ascertain their EO content, analyze factors influencing extraction, and study the effects of different pretreatment methods.

Prior to supercritical $CO_2$ extraction, preprocessing the materials can effectively enhance the efficiency of EO extraction. Common preprocessing methods include passive techniques such as ultrasonic treatment [9] and immersion in high-concentration solutions, as well as active methods like enzymatic solutions. Passive methods exploit the differential osmotic pressure inside and outside the cell to cause cell rupture, thereby increasing the extraction rate of EOs when carried by supercritical $CO_2$ fluid. Wu Y et al. [10] investigated the pretreatment of rose petals with a salt solution, which resulted in a doubled yield of bitter EOs compared to direct extraction., Enzymes react with plant cell walls, exposing the cell matrix [11]. Shende D et al. [11] utilized an enzymatic solution for oil extraction from corn germ, demonstrating higher total phenol content compared to the control group using solvent extraction. Polmann G et al. [12] also employed an enzymatic solution for walnut oil extraction, achieving a 65.3% extraction rate with a peroxide value of 1.99.

The application of various preprocessing methods mentioned above has been lacking in specific research focused on pretreating Xinjiang Hetian rose petals. This study utilizes supercritical $CO_2$ extraction to obtain EOs from Xinjiang Hetian roses, investigating and analyzing the influencing factors of the extraction process. Building upon this foundation, the study further determines the impact of different concentrations of salt and enzyme solutions as preprocessing agents, analyzing the reasons behind these effects, that contribute to extracting essential oils from plants with lower oil content poses. By focusing on

Hetian rose petals, our study aims to optimize extraction techniques for plants with similar characteristics, potentially benefiting a broader range of botanicals with low oil yields.

The primary objectives of this study are as follows: (1) Conducting single-factor comparative experiments to research and analyze the effects of factors such as temperature and pressure on EO extraction from Xinjiang Hetian roses. (2) Optimizing experimental parameters and selecting the optimal conditions for supercritical $CO_2$ extraction of Xinjiang Hetian REOs. (3) After determining the optimal extraction conditions, conducting preprocessing on Xinjiang Hetian rose petals using salt and enzyme solutions with different concentrations, measuring the extraction rates, and analyzing the underlying reasons for the variations.

## 2. Materials and Methods

### *2.1. Materials and Equipment*

Hetian rose petals, sourced from the Hetian region in Xinjiang, were carefully selected, cleaned, and dried. Dry materials are necessary for consistent and sustainable experimental conditions, as well as for long-term storage and transportation. The standard inspection sieve was obtained from Shaoxing Shangyu Huafeng Hardware Instrument Co., Ltd. (Shaoxing, China). The supercritical $CO_2$ extraction equipment was procured from Jiangsu Nantong Maichuan Supercritical Co., Ltd. (Wuxi, China). The milling machine used in the process was acquired from Yongkang Hongtaiyang Mechanical and Electrical Co., Ltd. (Yongkang, China).

### *2.2. Chemical Reagents*

$CO_2$ gas with a purity of 99.9%, meeting food-grade standards, was supplied by Chonghong Gas Co., Ltd. (Langfang, China). Cellulase (with a purity of 99.5%) was stored in a dry powder state at low temperatures, while pectinase (with a purity of 99.0%) was preserved in glycerol under activation conditions set at 40 °C [11]. Both enzymes were obtained from Cangzhou Xiasheng Enzyme Biotechnology Co., Ltd. (Cangzhou, China). Ethanol with a purity of 99.5% was used and distilled water was employed in the experiments.

### *2.3. Statistical Analysis Program*

The design in this study was carried out using Design-Expert 13, employing the Central Composite Design (CCD) method for optimization and performing variance analysis on the experimental results. MATLAB12b software was used to code and calculate the empirical models and derivative formulas.

### *2.4. Hetian Rose Essential Oil Supercritical $CO_2$ Extraction*

An orthogonal experiment was designed using Design-Expert 13 software. The influencing factors included extraction pressure (A), extraction temperature (B), $CO_2$ flow rate (C), particle size (D), and co-solvent content (E). The extraction pressure ranged from 30 MPa to 40 MPa, extraction temperature from 35 °C to 45 °C, flow rate from 5 L/h to 15 L/h, and particle size from 0.8 mm to 1.2 mm. The solution configuration used 99.8% sodium chloride salt as raw material. The concentrations for salt solution pretreatment were set at 5%, 10%, and 20%, while enzyme solution concentrations were set at 2%, 5%, and 10%. To maintain the purity of the experimental results and avoid potential influences, the salt and enzyme solutions were not used in combination. Table 1 presents the levels of the design factors.

The working principle of supercritical $CO_2$ extraction is illustrated in Figure 1. High-pressure $CO_2$ cylinders supply the $CO_2$ through control valves, and low-pressure cylinders receive the return gas. $CO_2$ from the high-pressure gas cylinder is released at 6 MPa pressure. It undergoes purification and filtration in the purifier and filter. Gaseous $CO_2$ is then cooled to 5 °C through a coil for liquefaction before entering the $CO_2$ tank to ensure the working state of the high-pressure pump. Liquid $CO_2$ is pressurized by the three-phase plunger high-pressure pump to the preset pressure inside the extraction vessel. The outer wall of the extraction vessel utilizes a wrapped water bath circulation heating method. Both the external water tank and the $CO_2$ outlet of the extraction vessel are equipped with temperature controllers for monitoring. The $CO_2$ flow rate is adjusted by varying the power of the high-pressure pump.

**Table 1.** Orthogonal experimental factor design.

| Factor | Name | Units | Subtype | Minimum | Maximum | Median | Coded Low | Coded High |
|---|---|---|---|---|---|---|---|---|
| A | Pressure | MPa | Continuous | 30.00 | 40.00 | 35 | −1 | +1 |
| B | Temperature | °C | Continuous | 35.00 | 45.00 | 40 | −1 | +1 |
| C | $CO_2$ flow | L/h | Continuous | 5.00 | 15.00 | 10 | −1 | +1 |
| D | Particle size | mm | Continuous | 0.80 | 1.20 | 1.0 | −1 | +1 |
| E | Co-solvent | % | Continuous | 0.00 | 20.00 | 10 | −1 | +1 |

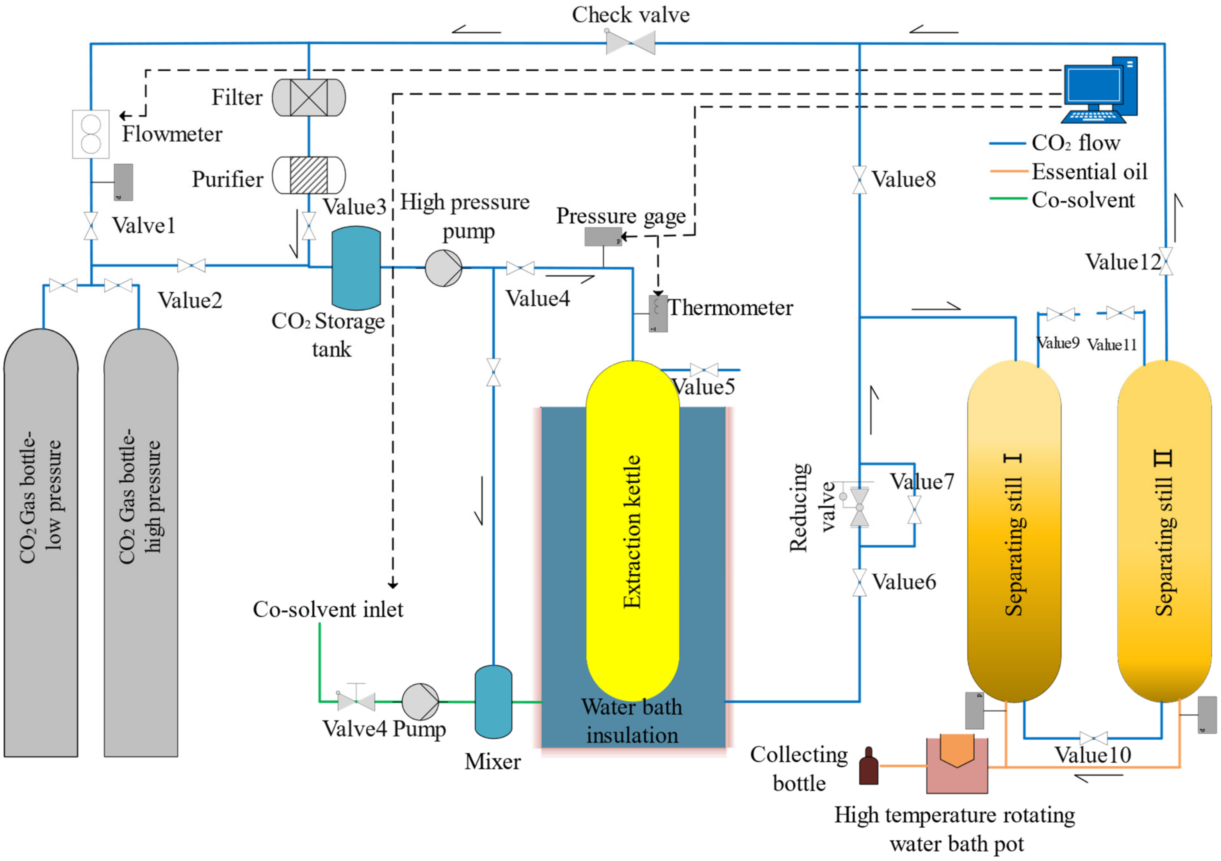

**Figure 1.** Supercritical $CO_2$ extraction process.

The supercritical fluid comes into contact with the material in the extraction vessel, carrying EO components. Through a manually operated back-pressure valve (with an error of ±0.01), the supercritical fluid undergoes depressurization separation within two-stage separation vessels. For safety, when changing materials, only the inlet and outlet valves of the extraction vessel are closed to release the gas, and the remaining majority of $CO_2$ is retained in the system or cylinders for future use.

The collected substance passes through a high-temperature rotary water bath at 70 °C [13], reducing the exposure time by taking advantage of ethanol's volatile nature and immediately cooled to minimize essential oil loss.

The EO is collected in a brown bottle. A co-solvent pump is added to the front end of the extraction vessel to introduce a co-solvent. After pressurization, the co-solvent is mixed with $CO_2$ in the mixer. The experiment lasts for 120 min, with collection occurring every 5 min.

The EO product is refrigerated at 0 °C for subsequent analysis and detection. Throughout the extraction process, parameters such as EO mass, $CO_2$ flow rate, extraction pressure, and temperature are recorded through data collection (Figure 2).

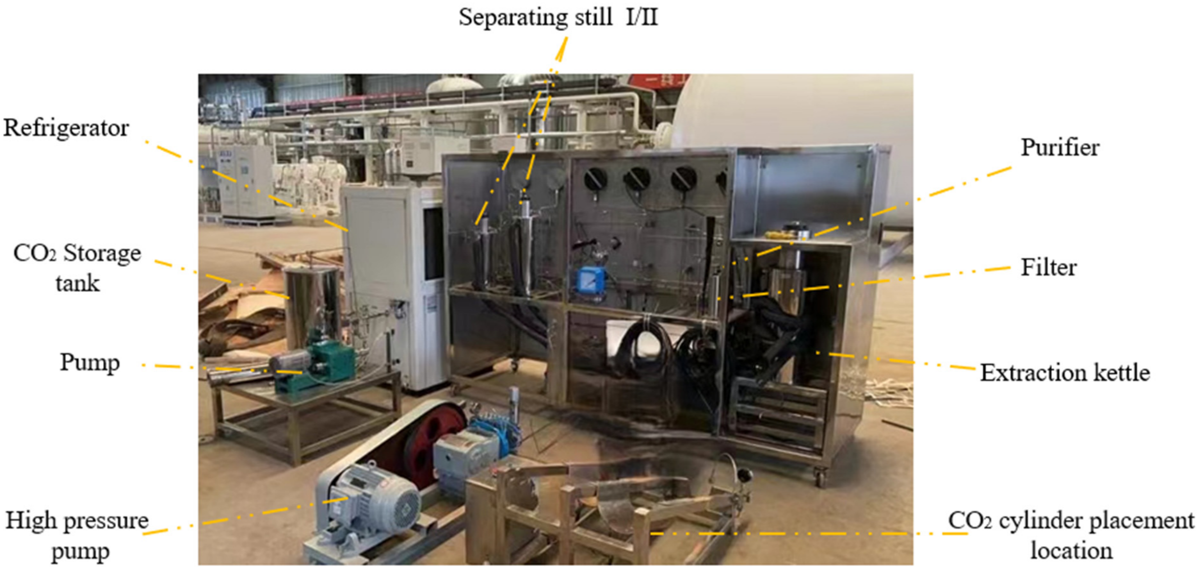

**Figure 2.** Physical image of extraction experimental platform.

The extraction rate of Xinjiang Hotan Rose EO is calculated as follows [14]:

$$y = \frac{m_1}{m} \times 100 \tag{1}$$

$y$ is the extraction rate of Hetian REOs, %; $m_1$ is the mass of EOs, g; and $m$ is the total mass of the material before extraction, g.

Treating EOs as a single component [15], the formula for calculating the solubility of EOs is

$$Y^*(H) = \frac{M^*}{M_{co_2}} H \tag{2}$$

$Y^*$ is the solubility of Hetian REOs; $M^*$ is the mass flow rate of the solute, kg/s; $M_{CO2}$ is the mass flow rate of the solvent, kg/s; and $H$ is the height of the extraction kettle, m.

## 3. Results and Discussion

Each experimental measurement was repeated three times. The experimental data were extracted from Hetian REOs under corresponding conditions until the stable stage with a change in EOs quality $\leq 0.1$ g.

### 3.1. Extraction Pressure and Temperature Effects

From Figure 3, it is evident that the extraction pressure significantly influences the extraction rate of Hetian REOs. Under the same conditions, the extraction rate at 35 MPa is 5.95%, higher than that at 30 MPa and 40 MPa. The solubility of Hetian REOs in $CO_2$ reaches its peak at a pressure of 35 MPa. This is because as the extraction pressure increases, the density of $CO_2$ fluid increases, leading to an elevation in solute solubility. At this point, the influence of pressure and temperature exhibits a positive effect. However, as the extraction pressure continues to rise to 40 MPa, the impact of pressure and temperature on the density of $CO_2$ fluid gradually turns negative, causing a reduction in solubility.

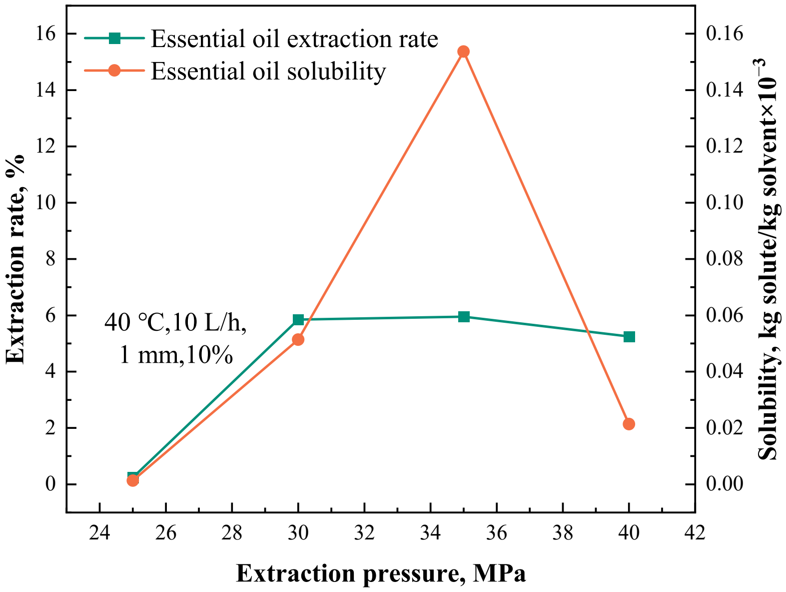

**Figure 3.** The influence of pressure on the extraction rate of essential oils.

Although increasing pressure enhances the density of $CO_2$, thereby increasing the fluid's solvency, excessively high pressure may induce local aggregation in some materials, leading to a decrease in EO solubility and a subsequent reduction in extraction rate. It is evident that excessively high extraction pressure does not necessarily further improve the extraction efficiency. Similar conclusions were drawn in the experiment on the extraction of pine terpenoids conducted by Rahimi-Nasrabadi M's team [16], where the optimal condition was found to be 10 MPa rather than 20 MPa or 30 MPa.

As shown in Figure 4, the EO extraction rate first rises and then declines with the increase in extraction temperature. Under the conditions of 40 °C, the EO extraction rate is relatively high, reaching 5.9%. The solubility of EOs is at its maximum at 40 °C. This is because raising the temperature can enhance molecular movement rates and also has a certain impact on the density of $CO_2$ fluid. However, excessively high temperatures can lead to the decomposition of some EO components, resulting in a decrease in the extraction rate [17].

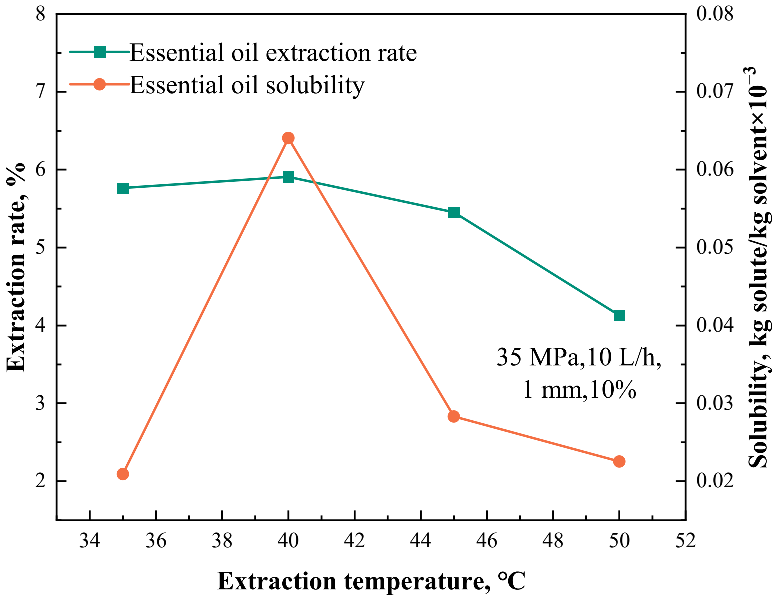

**Figure 4.** The effect of temperature on the extraction rate of essential oils.

The influence of temperature and pressure on the extraction rate is essentially the change in vapor pressure and fluid density. The effect of increasing pressure on the density of $CO_2$ is greater than the effect of temperature on the density of $CO_2$. As a result, the extraction rate increases, and the solubility of essential oil increases. This trend aligns with the findings of Edgar L et al. [18] in their soybean oil extraction experiment. When the combined effect of temperature and pressure shows a negative effect, the extraction rate decreases and the solubility of EOs decreases.

In summary, for supercritical $CO_2$ extraction of Hetian REOs, the positive effect range of pressure and temperature is observed at the conditions of 35 MPa and 40 °C. Under these conditions, the extraction rate is relatively high, and the extraction rate tends to plateau after 4 h of experimentation.

### 3.2. The Effect of Co-Solvents on Extraction Rate

As observed in Figure 5, the EO extraction rate at a 20% co-solvent content (15.54%) is higher than that at a 10% co-solvent content (5.95%) and the solubility of EOs shows a gradually increasing trend. The reason for this lies in the strong ability of $CO_2$ to dissolve non-polar components in the EOs under supercritical conditions. However, there is limited solubility for some polar bond substances, such as sesquiterpenoids, which exhibit poor solubility due to the presence of hydroxyl groups with strong polarity. Using ethanol as a co-solvent can enhance the solubility of polar substances, thereby increasing the overall EO extraction rate [18]. Similar conclusions were drawn by Yousefi M's team [13] in their experiments on supercritical $CO_2$ extraction of EOs.

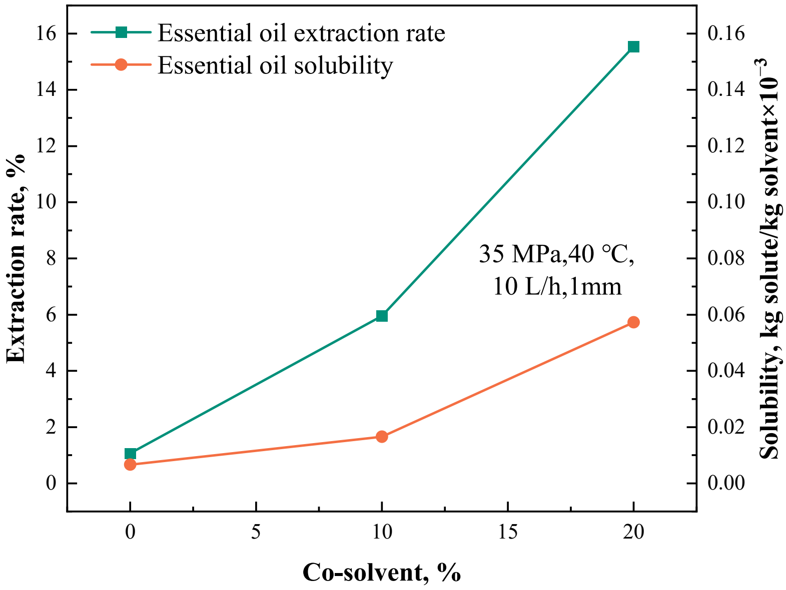

**Figure 5.** The influence of co-solvent content on extraction rate.

However, there is a significant difference of 9.58% in the extraction rates between 10% and 20% co-solvent contents. The reason for this difference is that, despite undergoing high-temperature rotary water bath treatment, some alcohol remains in the extraction product due to incomplete evaporation.

### 3.3. Analysis of Variance

The E (co-solvent) content shows a significant value of $p < 0.0001$, indicating a higher level of significance compared to A (pressure) and B (temperature). The significance value for extraction temperature is $p = 0.0344$, which is higher than that of extraction pressure ($p = 0.0636$). The quadratic term for temperature also demonstrates better significance compared to the other two factors. Empirical modeling of the above three influencing factors resulted in an overall significant model with $p < 0.0001$ (Table 2). The specific formula is as follows:

$$\begin{aligned} Yield = 6.14 - 0.8743A + 1.01B + 5.87E \\ -0.4267AB - 0.596AE + 0.4321BE- \\ 0.1891A^2 + 1.23B^2 - 0.1882E^2 \end{aligned} \tag{3}$$

**Table 2.** Analysis of variance.

| Source | Sum of Squares | df | Mean Square | F-Value | *p*-Value |
|---|---|---|---|---|---|
| Model | 545.59 | 9 | 60.62 | 17.4 | <0.0001 |
| A (Pressure) | 13.3 | 1 | 13.3 | 3.82 | 0.0636 |
| B (Temperature) | 17.73 | 1 | 17.73 | 5.09 | 0.0344 |
| E (Co-solvent) | 527.28 | 1 | 527.28 | 151.34 | <0.0001 |
| AB | 2.8 | 1 | 2.8 | 0.8048 | 0.3794 |
| AE | 5.16 | 1 | 5.16 | 1.48 | 0.2365 |
| BE | 2.71 | 1 | 2.71 | 0.7785 | 0.3872 |
| $A^2$ | 0.0988 | 1 | 0.0988 | 0.0283 | 0.8678 |
| $B^2$ | 4.16 | 1 | 4.16 | 1.19 | 0.2862 |
| $E^2$ | 0.0904 | 1 | 0.0904 | 0.0259 | 0.8735 |
| SD | | | 4.41 | | |

### 3.4. Uncertainty Calculation

For the uncertainty analysis of the experiment, the square-root method was used to calculate the uncertainty of A (pressure), B (temperature), and E (co-solvent) content as $\sigma_A \pm 0.1$ MPa, $\sigma_B \pm 0.5$ °C, and $\sigma_E$ 0.05%.

$$\sigma_{\text{Yield}} = \pm\sqrt{\left(\frac{dY}{dA}\sigma_A\right)^2 + \left(\frac{dY}{dB}\sigma_B\right)^2 + \left(\frac{dY}{dE}\sigma_E\right)^2} \tag{4}$$

Some of the derivatives are as follows.

$$\frac{dY}{dA} = -0.8743 - 0.4267B - 0.596E - 0.1891 \times 2A \tag{5}$$

$$\frac{dY}{dB} = 1.01 - 0.4267A + 0.4321E + 1.23 \times 2B \tag{6}$$

$$\frac{dY}{dE} = 5.87 - 0.596A + 0.4321B - 0.1882 \times 2E \tag{7}$$

The specific value $\sigma_{\text{Yield}}$ is calculated as ±1.15%.

### 3.5. The Effect of $CO_2$ Flow Rate on Extraction Rate

The $CO_2$ flow rate is also one of the factors influencing the extraction rate. As the fluid passes through plant cells, it dissolves EO components, and the speed of the flow directly affects the dissolution rate. A slow flow rate will prolong the extraction time, while a too-fast flow rate will result in the fluid being in an undersaturated state, inadequately dissolving the EOs, leading to poor extraction efficiency. Therefore, exploring an appropriate $CO_2$ flow rate is an effective means of improving the extraction rate (Figure 6).

The highest EO extraction rate is observed at a flow rate of 10 L/h, reaching 3.55%. With an increase in $CO_2$ flow rate, the solubility of Hetian rose EOs initially rises and then gradually decreases. This trend occurs because, with relatively stable extraction temperature and pressure, a faster flow rate enhances the carrying effect on EOs. Within a unit extraction time, a greater amount of EOs can be produced. However, the $CO_2$ flow rate indirectly affects the solubility of EOs. Excessive flow rates can prevent the supercritical fluid from reaching a saturated state, causing some solutes to remain in the extraction vessel, leading to a decrease in EO solubility. Similar phenomena were observed in the study conducted by Rodrigues V.M. [15] on cellulose solubility systems.

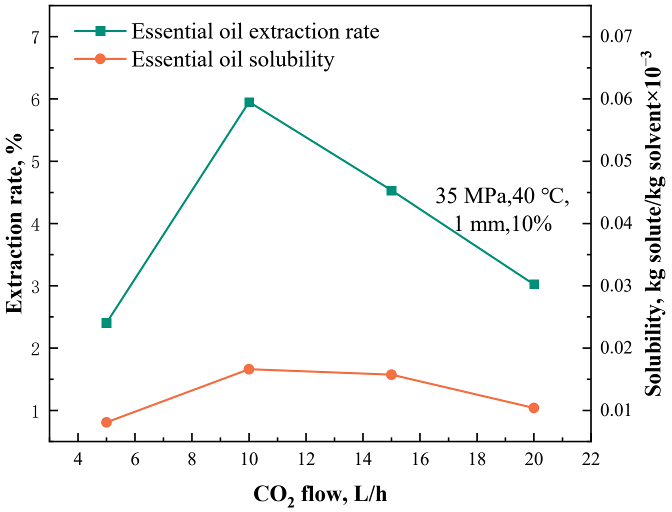

**Figure 6.** The effect of $CO_2$ flow rate on extraction rate.

### *3.6. The Effect of Particle Size on Extraction Rate*

The extraction rate of Hetian rose EOs is higher under the condition of a particle size of 0.8 mm compared to other particle sizes. The solubility at this particle size is 0.028 and the overall solubility of EOs is basically stable. Theoretically, smaller particle sizes achieved through pre-grinding of the material result in a larger contact surface area with the solvent, promoted the dissolution of essential oils and improve extraction efficiency (Figure 7).

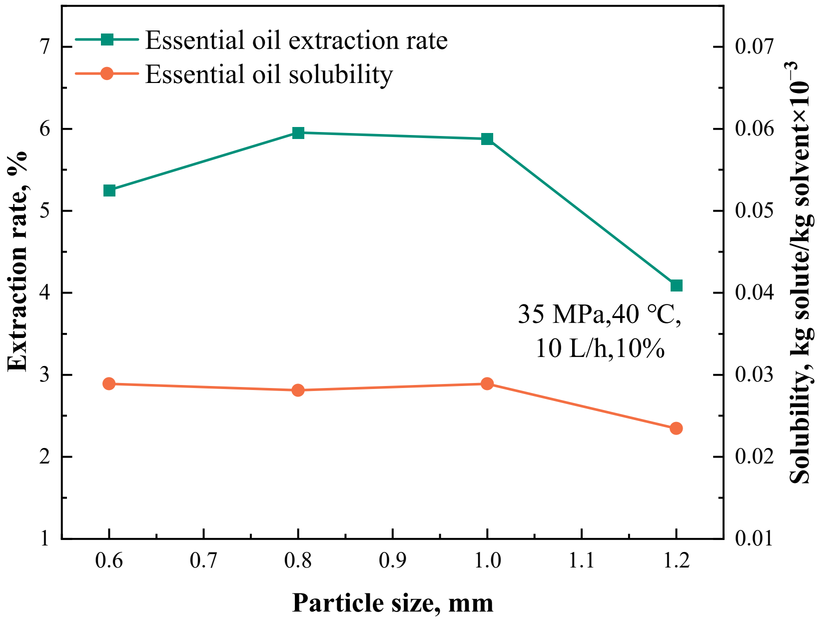

**Figure 7.** The influence of particle size on extraction rate.

Therefore, selecting an appropriate particle size is beneficial for enhancing the extraction rate. This finding is supported by the experiment conducted by Bozorgian A. et al. [18] on sunflower seed oil extraction. However, for Hetian rose petals with lower oil content, changes in solute solubility are not as pronounced. In cases of excessively high extraction pressure and extremely small particle sizes, some agglomerated materials may enter the pipeline, leading to blockages and a subsequent decrease in the extraction rate. Hence, selecting an appropriate material particle size is crucial [19].

## 4. Central Composite Design (CCD) Optimization

To reduce the number of repetitive experiments and simultaneously assess the impact of relevant factors, optimization methods were introduced, including Central Composite Design (CCD), Taguchi Design, and Box–Behnken Design (BBD). The Taguchi Design only considers major influencing factors and their interactions, lacking an assessment of interactions among higher-order factors. BBD requires input factors to be less than four; exceeding this limit can result in decreased calculation accuracy. On the other hand, CCD is suitable for three or more input factors, providing more precise results than BBD and Taguchi Design, with a sample capacity of 2k factorial and 2k + 2k + 1. This allows for better predictions from a smaller number of data points [13].

In this study, the CCD optimization method was employed with five influencing factors (extraction pressure, extraction temperature, material particle size, $CO_2$ flow rate, and co-solvent content) as input values. The objective function was to maximize the extraction rate within the parameter range. The optimal experimental conditions were predicted and the specific experimental data are presented in Table 3. Combining the results with single-factor comparative experiments, the optimum experimental conditions were determined to be 35 MPa, 40 °C, 10 L/h, 0.8 mm, and 20% co-solvent.

**Table 3.** Extraction efficiency of Hetian rose EOs under different conditions.

| Serial Number | Pressure (MPa) | Temperature (°C) | $CO_2$ Flow (L/h) | Particle Size (mm) | Co-Solvent (%) | Extraction Rate of Essential Oil (%) |
|---|---|---|---|---|---|---|
| 1 | 35 | 45 | 10 | 1 | 10 | 8.31 |
| 2 | 40 | 35 | 5 | 1.2 | 20 | 9.57 |
| 3 | 30 | 35 | 15 | 1.2 | 20 | 11.59 |
| 4 | 30 | 35 | 15 | 0.8 | 0 | 1.54 |
| 5 | 30 | 45 | 5 | 0.8 | 0 | 0.982 |
| 6 | 40 | 35 | 15 | 0.8 | 20 | 11.69 |
| 7 | 30 | 40 | 10 | 1 | 10 | 5.82 |
| 8 | 35 | 40 | 10 | 1 | 10 | 6.37 |
| 9 | 30 | 45 | 15 | 0.8 | 10 | 13.87 |
| 10 | 30 | 45 | 15 | 1.2 | 0 | 1.287 |
| 11 | 40 | 35 | 5 | 0.8 | 0 | 0.398 |
| 12 | 35 | 40 | 10 | 1 | 10 | 5.73 |
| 13 | 40 | 45 | 15 | 0.8 | 0 | 1.078 |
| 14 | 35 | 40 | 10 | 1 | 10 | 5.91 |
| 15 | 35 | 40 | 10 | 1.2 | 10 | 7.15 |
| 16 | 40 | 45 | 5 | 1.2 | 0 | 2.206 |
| 17 | 40 | 45 | 5 | 0.8 | 20 | 9.576 |
| 18 | 35 | 40 | 10 | 0.8 | 10 | 5.85 |
| 19 | 35 | 40 | 5 | 1 | 10 | 2.18 |
| 20 | 30 | 35 | 5 | 1.2 | 0 | 1.017 |
| 21 | 35 | 40 | 10 | 1 | 10 | 7.5 |
| 22 | 35 | 40 | 15 | 1 | 10 | 6.74 |
| 23 | 35 | 40 | 10 | 1 | 10 | 4.98 |
| 24 | 35 | 40 | 10 | 1 | 20 | 15.54 |
| 25 | 30 | 45 | 5 | 1.2 | 20 | 14.72 |

**Table 3.** *Cont.*

| Serial Number | Pressure (MPa) | Temperature (°C) | $CO_2$ Flow (L/h) | Particle Size (mm) | Co-Solvent (%) | Extraction Rate of Essential Oil (%) |
|---|---|---|---|---|---|---|
| 26 | 40 | 40 | 10 | 1 | 10 | 5.416 |
| 27 | 40 | 45 | 15 | 1.2 | 20 | 13.63 |
| 28 | 35 | 40 | 10 | 1 | 10 | 6.12 |
| 29 | 40 | 35 | 15 | 1.2 | 0 | 0.436 |
| 30 | 30 | 35 | 5 | 0.8 | 20 | 12.2 |
| 31 | 35 | 35 | 10 | 1 | 10 | 5.76 |
| 32 | 35 | 40 | 10 | 1 | 10 | 5.95 |

Figure 8 illustrates the comprehensive impact of extraction pressure and temperature on the extraction of Hetian rose EOs. Clearly, the increase in extraction temperature has a more significant effect on improving extraction efficiency compared to extraction pressure. The combined effect of both factors primarily determines the extraction rate of Hetian rose EOs. It is not necessarily the case that higher pressure and temperature always lead to better results; rather, it depends on the inherent characteristics of the materials. This phenomenon was also observed by Rajput S. [20] in their extraction experiments with kinnow peels. They found that the extraction rate reached 1.55% at 225 bar and 43 °C, but beyond these pressure and temperature conditions, the extraction rate decreased, providing strong support for the conclusion mentioned above.

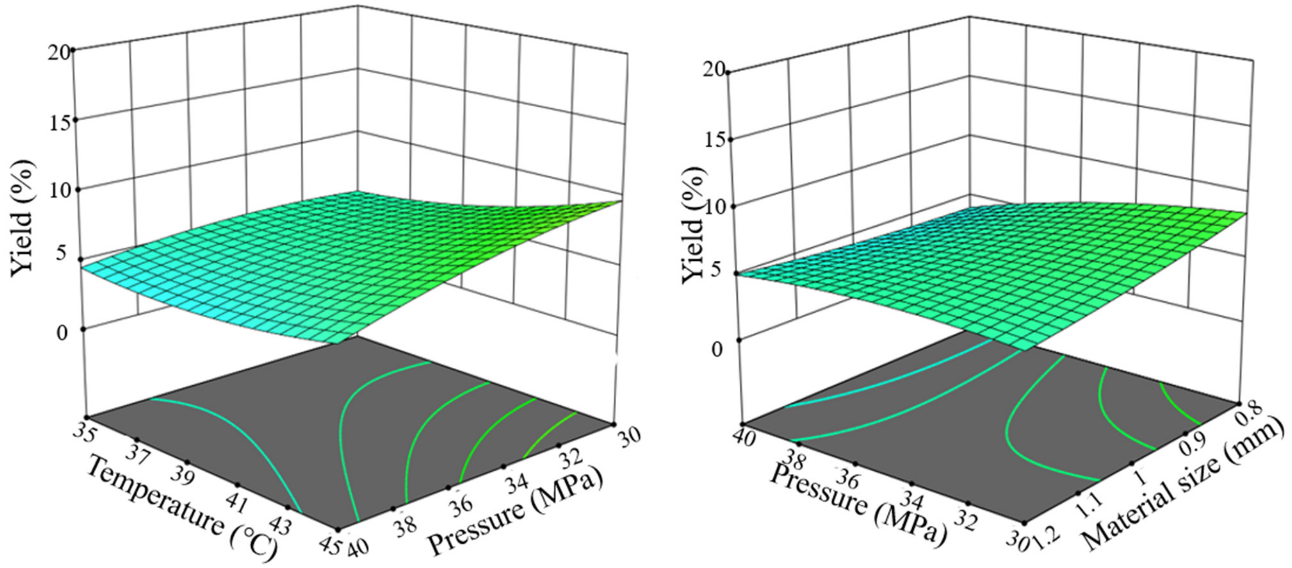

**Figure 8.** The comprehensive effects of pressure and temperature and pressure and particle size on the extraction rate of Hetian rose essential oil.

Increasing pressure has a noticeable effect on the extraction efficiency, especially for smaller particle sizes. However, when the pressure is too high, some materials may aggregate, causing blockages in the pipelines and leading to a decrease in extraction efficiency. Therefore, choosing an appropriate particle size is of crucial importance for improving extraction efficiency, as observed in the study by Putra, N.R. et al. [21], which reported similar phenomena.

Figure 9 illustrates the combined impact of particle size and $CO_2$ flow rate. The EOs are dissolved and entrained by the supercritical $CO_2$ fluid. Under the condition of smaller particle size, increasing the $CO_2$ flow rate can result in a higher yield of EOs. However, excessively fast flow rates can shorten the contact time between the fluid and the EOs, leading to a state where the supercritical fluid is not saturated, thus reducing the extraction efficiency. R A de Almeida et al. [22], through CFD simulation, explained the relationship between flow rate and average particle size. At the lowest flow rate, increasing the flow rate can reduce the average particle size (MPD) by 11.2%. However, at the highest flow rate, further increasing the flow rate leads to a 13.5% increase in the average particle size, confirming the above conclusion.

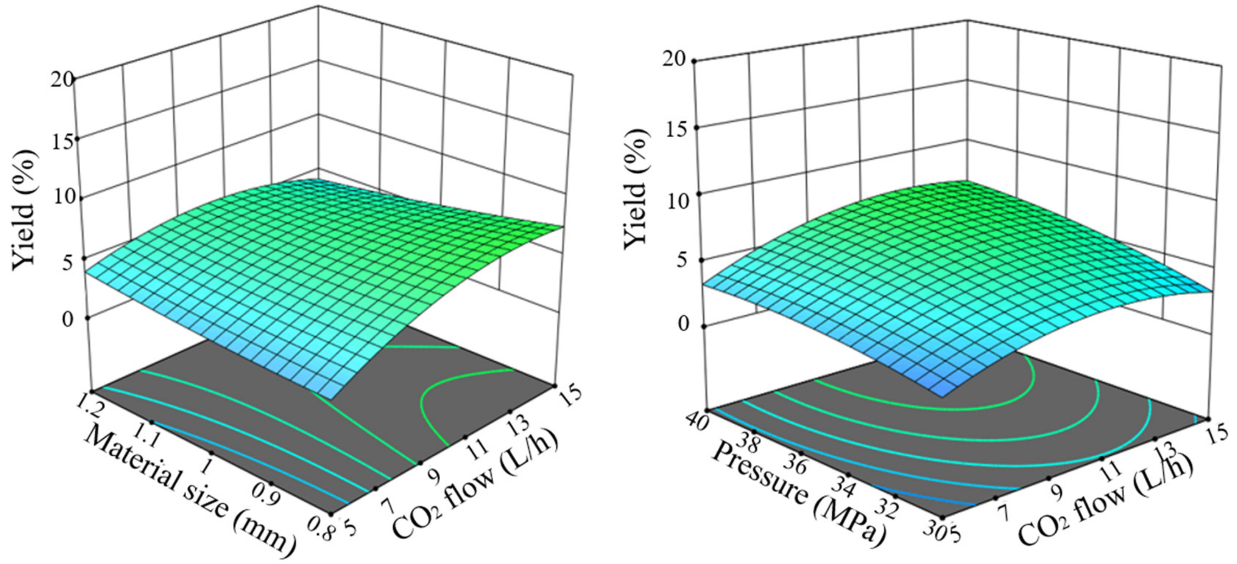

**Figure 9.** The comprehensive effects of $CO_2$ flow rate and particle size, pressure, and flow rate on the extraction rate of Hetian rose essential oil.

The combined effect of pressure and $CO_2$ flow rate on the extraction efficiency of Hetian rose EOs is shown in the central point of the surface. Increasing the extraction pressure can enhance the dissolution characteristics of the EOs. Under this condition, appropriately increasing the $CO_2$ flow rate can improve the extraction efficiency and increase the yield of Hetian rose EOs.

## 5. Effects of Salt Enzyme Pretreatment

Under the optimal conditions for supercritical $CO_2$ extraction of Hetian rose EOs, the study investigates the influence of different concentrations of salt and enzyme solutions on the oil extraction rate. Experiments were conducted using 2%, 5%, and 10% enzyme solutions and 5%, 10%, and 20% salt solutions.

To minimize the introduction of other mediums into the EOs, avoid influencing enzyme activity, and maintain oil purity, the addition of a co-solvent was omitted. This research focuses on the impact of different concentrations of salt and enzyme solutions on the extraction of Hetian rose EOs [23] (Figure 10).

The results indicate that the extraction rate is highest with a 10% salt solution, reaching 8.99%, while the extraction rate is lowest with a 20% salt solution, at 4.21%. The use of a 10% salt solution for assisting extraction results in greater solute solubility compared to 5% and 20% salt solutions. This is due to the increased saturation of the solution, leading to elevated osmotic pressure between cells. Consequently, plant cells swell and rupture, releasing the cellular matrix. This aligns with the findings of the study conducted by Yi Wua's team [10], where a salt solution was used for rose EO distillation. However, excessively high osmotic pressure might cause cell shrinkage without rupture, while too-low osmotic pressure may only lead to cell expansion without rupture. Therefore, the extraction rate with a 20% salt solution is lower than that with a 10% salt solution. Salt solutions exhibit excellent chemical stability and remain effective under supercritical $CO_2$ extraction conditions without requiring specific conditions to maintain their activity. In contrast, enzymes can deactivate or denature under certain specific conditions. Moreover, the sensitivity of enzymes to $CO_2$ under high-pressure conditions is not well-defined, which could potentially lead to decreased enzyme activity.

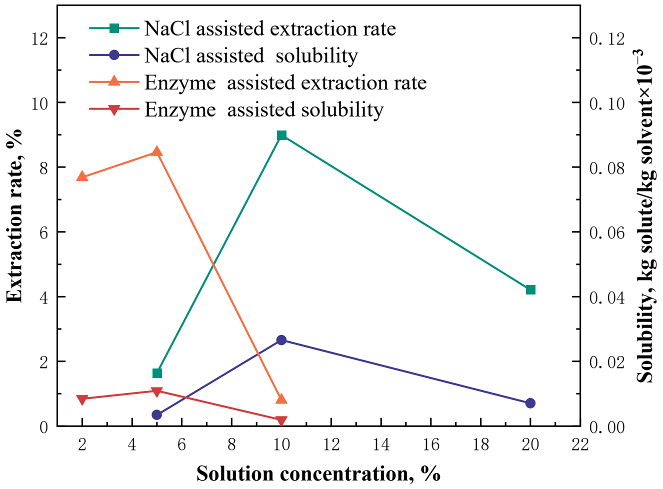

**Figure 10.** Extraction rate and solubility of different solution concentrations.

In comparison, the extraction rate with a 5% enzyme solution (8.46%) is 0.77% higher than with a 2% enzyme solution (7.96%). The solute solubility with a 5% enzyme solution is also greater than with 2% and 10% enzyme solutions. This is because both cellulose and pectin are fundamental substances forming the cell wall. The enzyme activation facilitates the active dissolution of the cell wall, releasing the cellular matrix. Similar results were observed in the experiment conducted by Lenucci M.S.'s team [24], where enzymatic extraction of tomato lycopene resulted in approximately three times higher yield compared to the control group. However, high-concentration solutions may cause some materials to agglomerate, reducing the contact area between the enzyme and cells, and leading to a decrease in extraction rate. Additionally, an enzyme solution may have a degrading effect on lipid substances like plant wax, indirectly enhancing EO yield [25]. Xiaoman Z's team [26] found in her research that the outer wall of plants has a cuticle layer, which contains cutin, a polyester. Cutinase can be used for hydrolyzing this target. In the research conducted by Sun R.'s team [27], cellulase was used to treat the outer wall of cherry tomatoes. Observations from a microscopic perspective revealed changes in the plant's cuticle, proving that enzymes may have potential effects on lipid substances.

Utilizing the immiscibility of essential oil and water, separation can be achieved by allowing the mixture to stand in a separatory funnel post-extraction. Subsequently, low-temperature treatment can be employed to crystallize the salt based on the relationship between solubility and temperature, followed by filtration. Due to the potential reactions between enzymes and essential oils, which may produce hydrolysis products, using chemical reagents for treatment would introduce new impurities. Therefore, no treatment was applied [28].

## 6. Conclusions

This experimental study investigated the supercritical $CO_2$ extraction of rose EOs from Hetian, Xinjiang. A comprehensive analysis was conducted to explore various factors influencing the extraction process, including extraction pressure, temperature, and co-solvent content. The optimal extraction conditions were determined through CCD optimization. Additionally, the solubility theory was applied to validate the obtained results. The efficiency of extracting Hetian rose with pretreatment using salt (5%, 10%, 20%) and enzyme solutions (2%, 5%, 10%) was measured. Specific conclusions are as follows:

(1) Temperature has a more significant impact compared to pressure, and co-solvent content shows great significance. However, due to the relatively low oil content in Hetian rose petals, reducing the particle size only provides limited assistance in improving extraction efficiency. Through CCD optimization techniques, the optimal conditions for extracting Hetian rose essential oil were determined as 35 MPa, 40 °C, 10 L/h, 0.8 mm particle size, and 20% co-solvent.

(2) Preprocessing Hetian rose materials is crucial for releasing essential oil components from plant cells; particularly important is controlling the concentration of salt solution used during this process. Although pectinase and cellulase have catalytic effects on essential oil extraction processes; high concentrations of enzyme solutions can lead to local micro-aggregation within materials, which hinders internal mass transfer processes. The highest extraction rate achieved was observed when using a salt solution concentration of 10%, resulting in an extract yield percentage of approximately 8.99%. Conversely, the lowest yield percentage (4.21%) was obtained when employing a salt solution concentration of 20%.

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## Abbreviations

| | | |
|---|---|---|
| $y$ | [%] | Extraction rate of Hetian rose essential oil |
| $m_1$ | [g] | Quality of essential oils |
| $m$ | [g] | Total mass |
| $Y^*$ | [kg solute/kg solvent] | Solubility |
| $M^*$ | [kg/s] | Mass flow rate of solutes |
| $M_{CO2}$ | [kg/s] | Mass flow rate of solvent |
| $H$ | [m] | Extraction kettle height |
| $\sigma_{Yield}$ | [-] | uncertainty |
| $\sigma_A$ | [-] | Pressure uncertainty |
| $\sigma_B$ | [-] | Temperature uncertainty |
| $\sigma_E$ | [-] | Co-solvent uncertainty |

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