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Optimization of Supercritical Extraction of Cannabidiol Using Response Surface Methodology

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Abstract: Hemp, also known as *Cannabis sativa* L., contains over 80 cannabinoids, with cannabidiol (CBD) being the primary neuroactive component. CBD possesses various pharmacological properties and is considered a non-psychoactive compound, making it a promising component for various applications, such as pharmaceuticals, cosmetics, and nutraceuticals. The aim of this study was to identify the optimal conditions for extracting CBD from hemp using supercritical fluid extraction (SFE). Response surface methodology (RSM) was employed to optimize the SFE conditions. The Box–Behnken design and the central composite design were utilized to refine the extraction parameters, including extraction time, temperature, and pressure. The statistical significance and reliability of the optimized conditions were confirmed by the significant influence of these independent variables on CBD yield. The extracted CBD was purified to a high level of purity and converted from cannabidiolic acid (CBDA) through heat treatment and then analyzed using high-performance liquid chromatography (HPLC). The following extraction conditions were considered optimal and led to a CBD yield of approximately 70.46 g/kg: pressure of 48.3 MPa, temperature of 60 °C, and extraction time of 109.2 min. Validation experiments confirmed the accuracy of the model, with experimental values closely matching the predicted values (69.93 ± 0.88 g/kg). This study demonstrates that SFE is an efficient method for obtaining high-purity CBD from hemp, highlighting its potential for industrial applications. The findings suggest that optimizing SFE conditions through RSM can significantly enhance the efficiency and yield of CBD extraction, providing a robust framework for industrial-scale production.

Keywords: *Cannabis sativa*; supercritical fluid extraction; Box–Behnken design; central composite design; cannabidiol extraction



Citation: Hong, M.; Kim, J.-H.; Kim, C.-H.; Lee, S.-U.; Kwon, T.-H.

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Processes **2024**, *12*, 2498. <https://doi.org/10.3390/pr12112498>

Academic Editor: Mustafa Mortas

Received: 14 October 2024

Revised: 1 November 2024

Accepted: 5 November 2024

Published: 11 November 2024



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1. Introduction

Hemp, scientifically known as *Cannabis sativa* L., has been cultivated worldwide for centuries, serving various purposes, ranging from industrial to medicinal uses. Its pharmacological potential has been recognized since ancient times, with its use documented in the earliest Chinese pharmacopeia, Shen Nung Pen Ts'ao Ching [1,2]. In traditional Chinese medicine, hemp was utilized for its therapeutic properties, including its use as an analgesic and anti-inflammatory agent [3]. Recent research has reignited interest in hemp, particularly because of its primary non-psychoactive cannabinoid, cannabidiol (CBD), which possesses various therapeutic properties [4,5]. In recent years, the global hemp industry has experienced significant growth due to increasing demand for its derivatives, particularly cannabinoids like cannabidiol (CBD) and tetrahydrocannabinol (THC). As of 2023, global hemp production reached approximately 310,000 tons, driven by rising interest in its medical and industrial applications. Furthermore, the economic value of the THC market was estimated at USD 43.2 billion in 2023, with a projected compound

annual growth rate (CAGR) of 17.5% through 2030. These figures highlight the importance of optimizing cannabinoid extraction processes for both medicinal and industrial uses [6]. Unlike tetrahydrocannabinol (THC), CBD does not induce psychoactive effects, making it an attractive candidate for medicinal and industrial applications [7,8].

The extraction of cannabinoids from hemp has traditionally involved the use of organic solvents, which pose environmental and health risks [9]. Organic solvents like ethanol and butane, although effective, can leave harmful residues and are not environmentally sustainable [10]. Supercritical fluid extraction (SFE) using CO₂ has emerged as a superior alternative owing to its efficiency and environmental benefits [11,12]. SFE is particularly advantageous as it allows for the extraction of high-purity cannabinoids without using harmful solvents [13]. This method uses CO₂ in its supercritical state, where it exhibits properties of both a liquid and a gas, enhancing the solubility of cannabinoids [14].

Optimization of the SFE process is crucial for maximizing CBD yield and purity. Liza et al. [15] explored the applications and challenges of supercritical fluid extraction for bioactive compounds, highlighting its potential to enhance extraction outcomes. Additionally, Chemat et al. [16] emphasized the importance of green extraction techniques, such as RSM, to optimize extraction conditions sustainably. RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It aids in evaluating the effects of several independent variables and their interactions on dependent variables [17]. For example, Gontard et al. [18] and Zaharia et al. [19] demonstrated that RSM effectively optimizes various extraction processes by systematically evaluating key variables and their interactions, thereby significantly improving the yield and quality of the desired compounds.

The objective of this study was to determine the optimal conditions for extracting CBD from hemp using SFE. By employing RSM, we systematically investigated the effects of extraction time, temperature, and pressure on CBD yield [20]. Specifically, the Box–Behnken design and central composite design were utilized to refine the extraction parameters [21]. These designs are robust tools in RSM that allow for the efficient exploration of the process variables, leading to the determination of optimal conditions [22,23]. The statistical significance and reliability of the optimized conditions were validated through experimental data, ensuring the robustness of the model [24].

By systematically investigating these variables, we aimed to maximize the yield and purity of CBD, providing a robust framework for the large-scale, sustainable, and eco-friendly production of hemp-derived CBD for various industrial applications.

2. Materials and Methods

2.1. Experimental Process

SFE was conducted to extract CBD from hemp (*Cannabis sativa* L.). RSM was applied to optimize the extraction conditions, employing a Box–Behnken design to adjust extraction time, temperature, and pressure. The extracted CBD was converted from cannabidiolic acid (CBDA) through heat treatment and analyzed using high-performance liquid chromatography (HPLC). Data analysis was performed using the Minitab software version 16 (Minitab Inc., State College, PA, USA), and optimal conditions were derived and validated [25].

2.2. Hemp Preparation

The hemp (*Cannabis sativa* ssp. *sativa*) used in this study was obtained from CFH Bioscience Ltd. (Longmont, CO, USA) and consisted of the Cherrywine variety's flower parts. It was dried using a hot air dryer (Daedong, Nonsan, Republic of Korea) at 40 °C for 48 h. After drying, it was ground to an 80-mesh size using a grinder (DA280-S; Daesung Artlon, Paju, Republic of Korea) and stored in a desiccator at 23 °C and a humidity level of 14% [26].

2.3. RSM Design

2.3.1. Experimental Design

To optimize the SFE method, an experimental design was conducted using the Minitab software (State College, PA, USA). The Box–Behnken design, a type of RSM, was employed [27]. The independent variables for the extraction conditions were encoded into three levels (−1, 0, 1) based on a preliminary experiment. These variables included extraction time (t, 60–120 min), extraction temperature (T, 35–70 °C), and extraction pressure (P, 20–50 MPa). Then, extraction was performed using a supercritical extractor.

2.3.2. Result Analysis

The dependent variables, extraction yield, and CBD content were measured and used in the regression analysis. Fifteen different experimental conditions based on the independent variables were tested (Table 1). The regression equations for the dependent variables showed R^2 values > 0.93, indicating values close to 1. This indicated the high reliability of the response models. Additionally, to investigate the influence of each dependent variable with respect to two of the three independent variables, the remaining variable was fixed at intermediate values: an extraction time of 90 min, extraction temperature of 52.5 °C, and extraction pressure of 35 MPa.

Table 1. Experimental values of cannabidiol (CBD) extraction yield based on extraction time, pressure, and temperature using the Box–Behnken design.

Experiment No.	Pressure (MPa)	Temperature (°C)	Time (min)	CBD Yield (g/kg in Raw Material ¹)
1	50	70	90	50.15
2	20	35	90	13.90
3	35	70	60	42.55
4	35	52.5	90	59.61
5	35	35	120	8.63
6	50	35	90	55.22
7	35	52.5	90	60.12
8	20	70	90	56.54
9	35	52.5	90	59.99
10	35	35	60	12.98
11	20	52.5	60	36.49
12	50	52.5	120	61.37
13	35	70	120	69.47
14	50	52.5	60	35.46
15	20	52.5	120	28.32
Optimal conditions:				
	47.27	60.45	105.45	68.25

¹ The content of ingredients contained in 1000 g of raw material.

2.3.3. Optimization

The range of the independent variables was narrowed to optimize the results using a central composite design. The variables were encoded into five levels (−1.682, −1, 0, 1, and 1.682), representing extraction pressure (42–52 MPa), extraction temperature (55–65 °C), and extraction time (100–110 min). Twenty experimental conditions of the independent variables were tested (Table 2). The regression equation for the dependent variable showed an R^2 value > 0.93, indicating the high reliability of the response model. Furthermore,

to understand the impact of two of the three independent variables on each dependent variable, the remaining variable was fixed at the midpoint values: extraction time at 105 min, extraction temperature at 60 °C, and extraction pressure at 47 MPa.

Table 2. Experimental values of cannabidiol (CBD) extraction yield based on extraction time, pressure, and temperature using a central composite design.

Experiment No.	Pressure (MPa)	Temperature (°C)	Time (min)	CBD Yield (g/kg in Raw Material) ¹
1	47	51.59	105	53.97
2	38.59	60	105	49.3
3	55.41	60	105	59.55
4	42	55	110	60.78
5	52	65	100	52.77
6	47	60	105	68.34
7	47	60	105	68.52
8	47	60	105	67.98
9	47	60	105	69.12
10	47	60	105	68.33
11	42	65	110	59.01
12	52	55	100	53.8
13	47	60	96.59	53.87
14	52	55	110	65.11
15	42	65	100	51.63
16	42	55	100	53.99
17	52	65	110	61.02
18	47	60	113.41	68.14
19	47	60	105	68.59
20	47	68.41	105	62.02
Optimal conditions:				
	48.27	60.00	109.16	70.45

¹ The content of ingredients contained in 1000 g of raw material.

2.4. Extraction and Analysis

2.4.1. SFE Procedure

A dried cannabis sample of 1000 g was placed in a 10 L supercritical extraction vessel and sealed to prevent gas from escaping. The extraction was performed using a supercritical extractor (SFE-CO₂-10L-40M-60C-R; Phosentech Co., Ltd., Daejeon, Republic of Korea) under the conditions specified in Tables 1 and 2, with CO₂ flow at 500 g/min, sampler temperature at 54 °C, and separator temperature at 30 °C. The collected extract was left at room temperature for approximately 2 h until all the CO₂ had vaporized. Subsequently, the weight of the extract was measured for further processing.

2.4.2. Decarboxylation

In its natural state, hemp predominantly contains CBDA rather than CBD within the plant tissues. Therefore, to convert CBDA to CBD, we conducted a decarboxylation process through heat treatment [28]. Briefly, the extract was heated at 130 °C while stirring for 60 min in an oven (DH.DryG0105; DaiHan Scientific, Wonju, Republic of Korea). Subsequently, we confirmed the complete conversion of CBDA into CBD through HPLC analysis.

2.4.3. HPLC Analysis

For HPLC analysis, the extracted samples were mixed with 70% ethanol to a final concentration of 10 mg/mL. Subsequently, the samples were subjected to sonication for 2 h and filtered through a 0.45 µm syringe filter. Purified CBD (Sigma-Aldrich, St. Louis, MA, USA) was diluted in ethanol and used as the standard. Vials containing 100 µL of the diluted CBD sample or standard were prepared for analysis. HPLC was conducted using the Prominence LC-20A Modular HPLC system (Shimadzu, Kyoto, Japan) with an Agilent ZORBAX Eclipse plus C18 (4.6 mm × 250 mm, 5 µm) reverse-phase column (Agilent Technologies, Inc., Santa Clara, CA, USA) at 35 °C. Detection was performed at a wavelength of 228 nm using a UV detector. The mobile phase was run in gradient mode with a flow rate of 1 mL/min: B% 0–4 min 76%, 4–12 min 83%, 12–17 min 95%, 17–20 min 95%, 20–21 min 76%, and 21–30 min 76%.

2.4.4. Data Analysis

For the analysis of CBD extraction optimization, we utilized the Minitab software to carry out the experimental design, data analysis, and obtain a second-order model with an analysis of variance (ANOVA). We applied the regression coefficients to the second-degree polynomial model using experimental values. Using the prediction equation of RSM, we determined the optimal conditions for CBD extraction based on extraction time, extraction temperature, and extraction pressure. The goodness of fit for the polynomial model was expressed using the coefficient of determination (R^2), and statistical significance was investigated using an F-test within the same program.

2.4.5. Prediction and Verification

Optimal extraction conditions were predicted within the overlapping ranges of the characteristics. We selected an arbitrary central point within the predicted range, and the predicted values were determined by substituting them into each regression equation. We validated the accuracy of the predicted values through actual experiments. Specifically, we conducted experiments using the predicted variable conditions and compared the predicted response values with the actual experimental values. The difference between the predicted and experimental values was expressed using the relative error.

2.4.6. Statistical Processing

All analyses were conducted in triplicate. Statistical processing was performed using the Minitab software, and significant differences within a 95% confidence interval were confirmed through one-way ANOVA.

2.5. CBD Purification

2.5.1. CBD Isolation

To purify the extract obtained through SFE into high-purity CBD, 99% ethanol was added at a ratio of 10 times the extract volume. Subsequently, the mixture was placed in an ultralow-temperature freezer (907-WC; Thermo Fisher Scientific, St. Louis, MA, USA) at −70 °C for 24 h. The solidified wax was separated using vacuum filtration with filter paper (Whatman® WF2-0900; Whatman, Maidstone, UK). The ethanol was evaporated through rotary evaporation (Rotavapor R-220SE, Büchi Labortechnik AG, Flawil, Switzerland) at 70 bar and 45 °C for 60 min. Subsequently, the concentrated extract was placed in a medium-pressure liquid chromatography (MPLC) system (Isolera™ ISO-1EV; Biotage, Uppsala, Sweden) equipped with a Biotage® Sfär C18 D column (FSUD-0401-0120; Biotage, Uppsala, Sweden). Ethanol (70%) in distilled water was used as the mobile phase at a flow rate of 50 mL/min to separate the CBD. Subsequently, the solvent was removed from the separated CBD using a rotary evaporator, and 70% pure CBD oil was obtained.

2.5.2. CBD Crystallization

To obtain CBD crystals with a purity of 99% from the 70% pure CBD oil, a crystallization process was employed. The CBD oil was dissolved in n-Heptane (H1146; Samchun Chemicals, Seoul, Republic of Korea) at 30 °C and stirred using a LabTech hotplate stirrer (LMS-1003; Labtech, Sorisole, Italy). It was then slowly cooled to −10 °C in a refrigerator for approximately 60 min. The resulting CBD crystals were separated using a vacuum filtration system (12079; ULVAC Technologies, Inc., Kanagawa, Japan) with filter paper (Whatman® Grade 2, 4.7 cm; Whatman). The purity of the obtained powdered CBD was measured using HPLC and nuclear magnetic resonance (NMR) spectroscopy (AVANCE™ III 400 MHz; Bruker, Billerica, MA, USA).

3. Results and Discussion

3.1. Experimental Design Based on Response Surface Analysis

3.1.1. Effects of Box–Behnken’s Independent Variables and Correlation with Applied Models for Hemp Extract Preparation

Various studies have shown that terpene compounds exhibit high solubility in supercritical fluids, significantly affecting the final yield during extraction under supercritical conditions [29]. To optimize the extraction conditions for the functional compound CBD in hemp and enhance its medicinal and dietary potential, we conducted experiments under 15 different conditions generated using the Box–Behnken design of response surface analysis. Table 1 presents the experimental values of CBD extraction yield based on three independent variables: extraction time (t, 60–120 min), temperature (T, 35–70 °C), and pressure (P, 20–50 MPa).

The lowest yield was observed in Experiment 5 (T: 35 °C; t: 120 min; and P: 35 MPa), yielding 8.63 g/kg. Conversely, the highest yield was observed in Experiment 13 (T: 70 °C; t: 120 min; and P: 35 MPa), yielding 69.47 g/kg. Notably, the extraction time and pressure for these two experiments were similar; however, the yield increased with an increasing temperature. Overall, there was a significant variation in yield depending on the extraction conditions, with CBD yields ranging from 8.63 to 69.47 g/kg.

The response values were analyzed using the Minitab software. The regression equation, indicating the impact of each independent variable on the CBD yield, was as follows:

$$Y = 59.91 + 8.369 P + 15.996 T + 5.039 t - 4.476 P^2 - 11.48 T^2 - 15.023 t^2 - 11.924 PT + 8.517 Pt + 7.818 Tt \quad (1)$$

Y represents the CBD yield (g/kg) as a function of the independent variables of time (t), temperature (T), and pressure (P). The equation $Y(t)Y(t)Y(t)$ was derived using the response surface methodology (RSM) by fitting a second-order polynomial model to the experimental data. This model includes linear, quadratic, and interaction terms for the main factors of extraction time, temperature, and pressure. Regression analysis was conducted to estimate the coefficients, minimizing residuals between the observed and predicted values to optimize the CBD yield. The final equation $Y(t)Y(t)Y(t)$ represents the predicted CBD yield based on the optimized values of these variables. The statistical processing of the results is presented in Supplementary Table S1, showing the coefficients and significance of the first-order (t, T, and P), second-order (t^2 , T^2 , and P^2), and interaction terms (tT, tP, and TP) for CBD yield. Among the first-order terms, extraction temperature (T) and pressure (P) had significant effects, with temperature being significant within 1% ($p < 0.01$) and pressure within 5% ($p < 0.05$). Among the second-order terms, extraction temperature (T^2) and extraction time (t^2) were significant within 5% ($p < 0.05$). Similarly, the interactions between extraction pressure and temperature (PT) and extraction pressure and time (Pt) had significant effects within 5% ($p < 0.05$). These results demonstrate the significance of the second-order regression model for CBD yield, with extraction temperature being the most influential factor. Additionally, the significant influence of pressure on CBD yield aligns with the principles of SFE, where an increased pressure enhances the solvent power of CO_2 ,

thereby improving the extraction efficiency. However, like temperature, excessively high pressures may lead to higher operational costs and equipment wear without proportional increases in yield, emphasizing the need for optimal pressure settings [30,31].

As the extraction time increases, the solubility and rate of terpene extraction, including CBD, increase [32]. This leads to higher yields, a trend which has been observed in other studies as well [33,34]. The significance of extraction temperature and time was evident in the square terms and their cross-interaction effects ($p < 0.05$), while no significance was observed in the square and interaction terms between other factors. The ANOVA results for the second-order model are presented in Supplementary Table S2. The regression model showed an F-value of 8.14, indicating a highly significant impact within 1%. Furthermore, the first-order terms had p -values within 1%, the second-order terms were highly significant within 5%, and the interaction terms were also statistically significant, with a p -value close to 5% ($p = 0.054$). Extraction time also plays a pivotal role in determining the yield. While longer extraction times generally result in higher yields owing to an extended contact time between the solvent and the plant material, diminishing returns are observed beyond certain durations. This suggests an optimal extraction time, beyond which the increase in yield does not justify the additional time and energy costs [35].

The coefficient of determination (R^2) and the adjusted R^2 were 0.936 and 0.821, respectively. This suggests that the second-degree polynomial equation explains 93.6% of the response in the tested conditions, indicating that the equation is suitable for predicting the final response values. Additionally, the lack of fit test resulted in a $p < 0.01$, signifying that the model was a good fit for explaining the response.

3.1.2. Response Surface Analysis of Hemp Extract

The significance of the second-order regression equation for the response variable based on the extraction conditions prompted further exploration of the second-order regression model using response surface plots and contour plots to determine the optimal extraction conditions. Figure 1A illustrates the CBD extraction yield when the extraction time was fixed at the central value of 90 min, depicting its dependency on extraction temperature and pressure. The CBD content in the hemp extract increased sharply with increasing temperatures and decreased at temperatures $> 65^\circ\text{C}$. This trend indicates that the increase in temperature enhances the solubility and diffusion rates of CBD into the supercritical CO_2 phase, facilitating efficient extraction. However, excessively high temperatures may degrade sensitive compounds or lead to the co-extraction of unwanted substances, thereby reducing the purity of the extract. This highlights the importance of optimizing temperature conditions to achieve maximum yield without compromising quality [36]. Similarly, the CBD yield increased as the extraction pressure increased and reached a plateau around 40–45 MPa before decreasing at higher pressures. While an increased pressure enhances the solvent power of CO_2 , excessively high pressures can result in higher operational costs and accelerate equipment wear. Therefore, identifying the optimal pressure level is crucial to ensure efficiency without unnecessary resource expenditure [37]. These results underscore the industrial efficiency of increasing temperature and pressure to optimal conditions for CBD extraction. Lower extraction yields were observed at low temperatures and pressures, suggesting that CBD, which is relatively thermally stable, is influenced by both variables [38]. Ultimately, the response surface and contour plots predicted maximum extraction conditions (CBD yield > 60 g/kg) at 20–50 MPa and 50–70 $^\circ\text{C}$ [39].

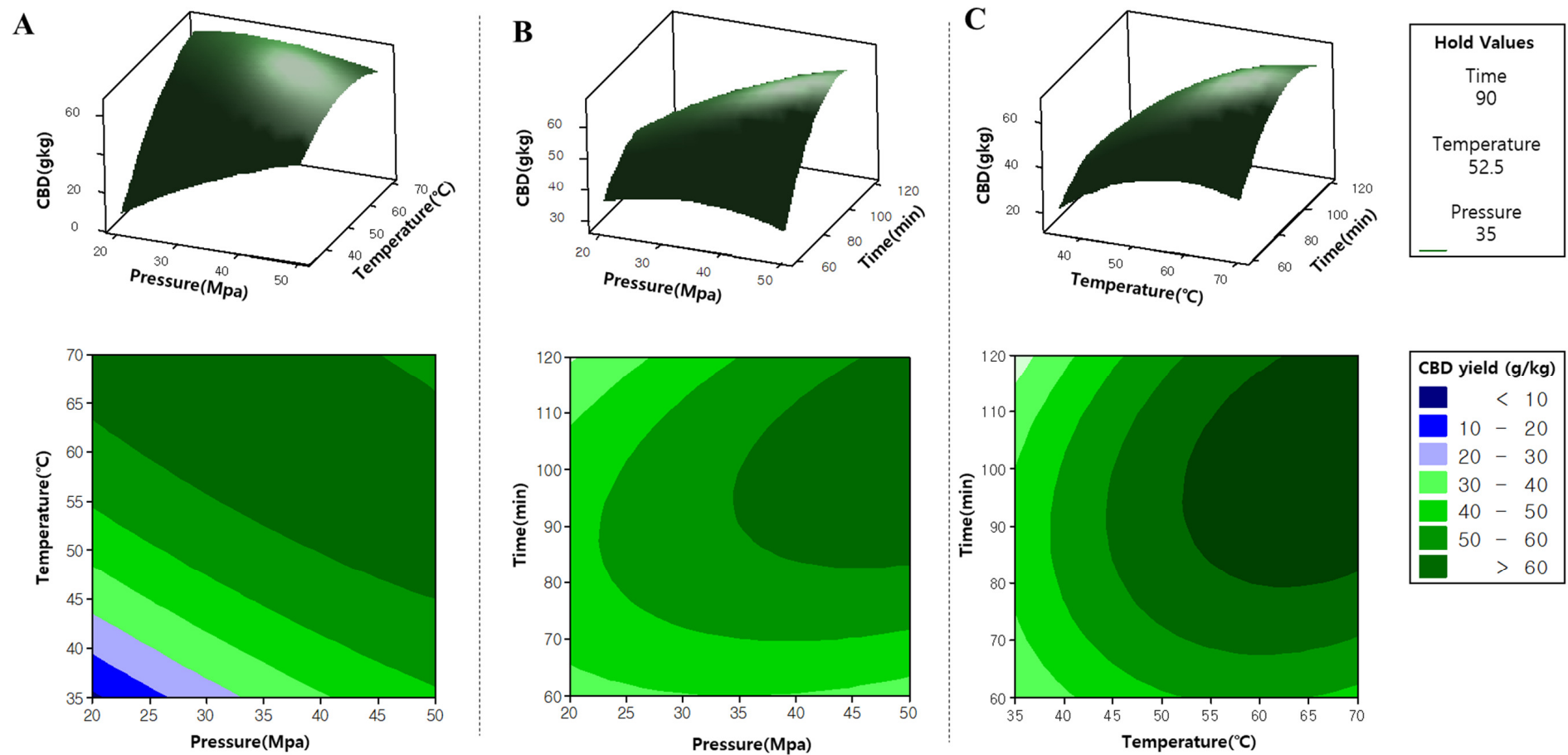


Figure 1. Response surface plots for cannabinoid (CBD) extraction yield at constant values of extraction time, temperature, and pressure: (A) CBD extraction yield as a function of extraction pressure and temperature, with time fixed at 90 min; (B) CBD extraction yield as a function of extraction time and pressure, with extraction temperature fixed at 52.5 °C; and (C) CBD extraction yield as a function of extraction time and temperature, with extraction pressure fixed at 35 MPa.

When the extraction temperature was fixed at the central value of 52.5 °C, CBD extraction yield as a function of extraction time and pressure showed maximum conditions at values of 37–50 MPa for pressure and 83–120 min for extraction time (Figure 1B). As the extraction time increased, the yield initially increased and then decreased around 100 min, suggesting an optimal extraction time beyond which the increase in yield was not significant enough to justify the additional time and energy costs [40]. Additionally, the extraction pressure increased gradually [41].

Figure 1C illustrates CBD extraction yield as a function of extraction time and temperature, with extraction pressure fixed at 35 MPa. The response surface plot and contour plot for the extraction yield appear to be nearly linear. The maximum extraction conditions were predicted to be at least 83 min for extraction time and 55 °C for extraction temperature.

3.2. CBD Extraction Process Optimization

3.2.1. Central Composite Design: Independent Variable Effects and Correlation with Applied Models for Hemp Extract Preparation

To optimize the extraction process using the Box–Behnken design, we conducted experiments based on 20 conditions using the central composite design. The experimental values for the CBD extraction yield, influenced by three independent variables—extraction time (*t*, 105–110 min), temperature (*T*, 55–65 °C), and pressure (*P*, 42–52 MPa)—are presented in Table 2.

The lowest yield was observed in Experiment 2 (*T*: 60 °C; *t*: 105 min; and *P*: 38.59 MPa), yielding 49.3 g/kg. Conversely, the highest yield was observed in Experiment 9 (*T*: 60 °C; *t*: 105 min; and *P*: 47 MPa), yielding 69.12 g/kg. These findings suggest that both temperature and pressure significantly influence the extraction yield, with higher values generally improving the yield up to a certain point. Beyond these optimal conditions, the yield may decrease due to the degradation of sensitive compounds or the co-extraction of unwanted substances, which is consistent with SFE principles [42]. Ultimately, the CBD content extracted from hemp ranged from 49.3 to 69.12 g/kg, indicating a significant variation in yield depending on the extraction conditions.

The response values were analyzed using the Minitab software. The regression equation, indicating the impact of each independent variable on the CBD yield, was as follows:

$$Y = 68.48 + 1.796 P + 0.314 T + 4.227 t - 4.948 P^2 - 3.685 T^2 - 2.621 t^2 - 0.124 PT + 0.674 Pt - 0.309 Tt \quad (2)$$

Y represents the CBD yield (g/kg) as a function of the independent variables of time (*t*), temperature (*T*), and pressure (*P*). The statistical analysis results are presented in Supplementary Tables S3 and S4, showing the coefficients and significance of the first-order (*t*, *T*, and *P*), second-order (*t*², *T*², and *P*²), and interaction terms (*tT*, *tP*, and *TP*) for CBD yield. Among the first-order terms, extraction time (*t*) and pressure (*P*) had significant effects within 1% (*p* < 0.01) and 5% (*p* < 0.05), respectively. This indicates that extraction time and pressure are critical factors influencing the CBD yield, likely owing to their effects on solubility and mass transfer rates [43]. In the second-order and interaction terms, it was observed that the extraction temperature (*T*²), extraction time (*t*²), and extraction pressure (*P*²) had significant effects within 1% (*p* < 0.01). These results underscore the importance of considering quadratic and interaction effects to fully understand and optimize the extraction process [44].

The coefficient of determination (*R*²) and the adjusted *R*² were 0.934 and 0.915, respectively. This suggests that the second-degree polynomial equation explains 93.4% of the variance in the tested conditions, indicating that the equation is suitable for predicting the final response values. A high *R*² value close to 1 indicates strong linearity and suggests a well-fitted model. However, a drawback arises as the *R*² tends to increase with an increase in the number of variables, which may not necessarily reflect the model's actual improvement. To address this, the adjusted *R*² was also considered. A minimal difference between the *R*² and the adjusted *R*² (<0.2) confirms the model's reliability. In this

study, all models demonstrated a minimal difference between the R^2 and the adjusted R^2 (<0.2), indicating a strong correlation and affirming the suitability of the response surface models [45]. Additionally, the lack of fit test resulted in a $p < 0.01$, signifying that the model was a good fit for the explanation.

These findings validate the robustness of the central composite design in optimizing the extraction process. The strong correlation and minimal difference between the R^2 and adjusted R^2 values affirm the model's accuracy in predicting outcomes under different extraction conditions, making it a reliable framework for industrial applications [46].

3.2.2. Response Surface Analysis of Optimal Hemp Extract

The quadratic regression equation of the optimized response variable was used to explore the form of the second-order regression model. Figure 2A illustrates CBD extraction yield as a function of extraction temperature and pressure, with the extraction time fixed at 105 min. The highest CBD content was observed within the temperature range of 55–65 °C and pressure range of 44–51 MPa. This indicates that both temperature and pressure are critical for maximizing the CBD yield, with optimal conditions within these specific ranges. Beyond these optimal points, the yield may decrease owing to the degradation of thermally sensitive compounds or inefficient extraction [47]. Furthermore, upon comparison between time and pressure, as well as time and temperature, the range for the maximum CBD yield narrowed, indicating a closer proximity to optimization. Ultimately, the response surface plot and contour plot for the extraction yield predicted that the maximum extraction conditions (CBD yield > 60 g/kg) would be within 47–49 MPa in terms of extraction pressure and a 59–61 °C extraction temperature.

Figure 2B illustrates the CBD extraction yield as a function of extraction time and pressure, with the extraction temperature fixed at 60 °C. The maximum extraction conditions were predicted to be within 44–51 MPa for extraction pressure and 107–111 min for extraction time. These findings suggest that extending the extraction time beyond this range does not significantly enhance the yield, thus highlighting the importance of identifying the optimal extraction duration to avoid unnecessary energy consumption and costs [48]. Finally, Figure 2C illustrates the CBD extraction yield as a function of extraction time and temperature, with extraction pressure fixed at 47 MPa. The response surface plot and contour plot for the extraction yield appeared as nearly circular, predicting maximum extraction conditions after 109 min for extraction time and at a 60 °C extraction temperature. These results suggest that maintaining an extraction temperature around 60 °C and optimizing the extraction time can significantly improve the efficiency and cost-effectiveness of the extraction process [49]. Additionally, excessive increases in extraction time and temperature in industrial extraction processes are unnecessary [50].

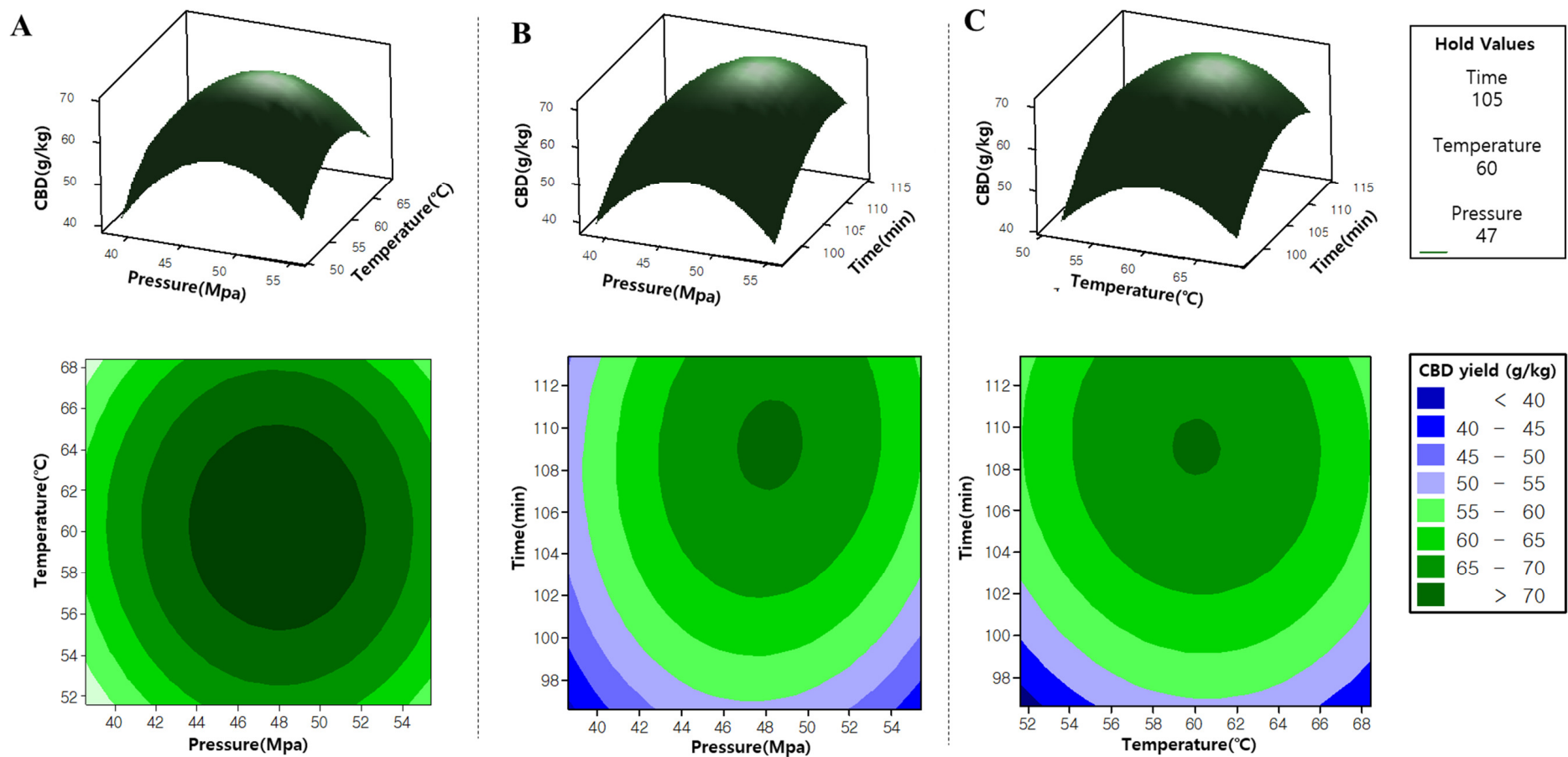


Figure 2. Response surface plots for cannabinoid (CBD) optimization at constant values as a function of temperature, pressure, and time: (A) CBD yield as a function of extraction temperature and pressure, with extraction time fixed at 105 min; (B) CBD yield as a function of extraction time and pressure, with extraction temperature fixed at 60 °C; and (C) CBD yield as a function of extraction time and temperature, with extraction pressure fixed at 47 MPa.

3.2.3. Hemp CBD Extraction Process Optimization

We optimized the extraction conditions for extracting CBD from hemp using RSM, considering the variables of extraction pressure, temperature, and time. The optimized conditions obtained from this study are shown in Figure 3: extraction pressure of 48.3 MPa, extraction temperature of 60 °C, and extraction time of 109.2 min. The predicted CBD yield under these optimal extraction conditions was 70.46 g/kg. Actual extraction experiments were conducted in triplicate using the predicted optimal conditions, yielding values of 69.93 ± 0.88 g/kg. The close agreement between the predicted and experimental values highlights the reliability and accuracy of the response surface model in real-world applications [51]. The accuracy of the model was verified by comparing the predicted extraction yield from the response surface analysis with the experimental values. The minimal error between the predicted and experimental yields was not significant, demonstrating the robustness of the optimization model. Thus, this model can be a valuable tool for process engineers aiming to enhance extraction efficiencies.

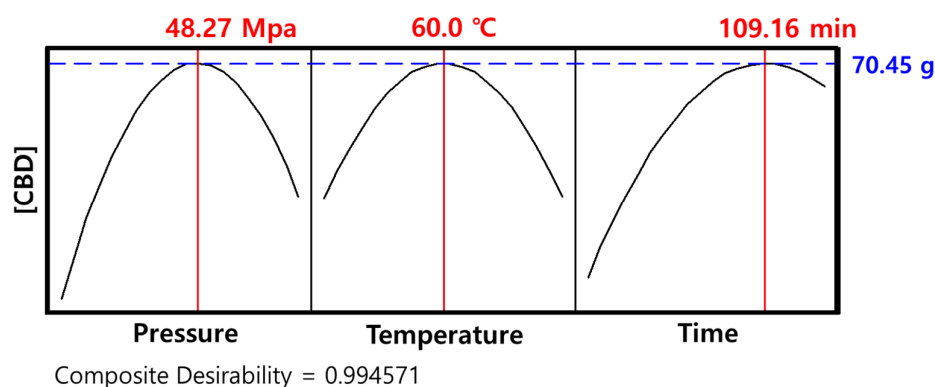


Figure 3. Response surface optimization graph of cannabidiol (CBD) yield.

Furthermore, the second-degree polynomial equations for each response variable in the response surface model were significant. Based on these findings, it was concluded that the optimized response surface model was suitable for explaining the response. The significance of the polynomial equations underscores the importance of considering quadratic terms in modeling to capture the complex relationships between the variables involved in the extraction process [52].

Additionally, the SFE process provides multiple advantages, including high extraction efficiency, reduction in harmful solvents, and alignment with sustainable practices. This method is especially advantageous for applications requiring high-purity CBD as it minimizes environmental hazards and solvent residues. Nevertheless, SFE does come with limitations, such as the high initial cost of equipment and significant energy demands to maintain optimal conditions, impacting cost-effectiveness for large-scale applications. Future improvements may involve combining SFE with complementary extraction methods to further enhance both yield and efficiency.

3.3. CBD Crystal

The oil extracted from hemp contains various terpenes and waxes in addition to CBD. To obtain high-purity CBD, a purification process is essential. Therefore, the oil fraction containing a high concentration of CBD (approximately 3–5 column volumes) was purified using MPLC, and the highest peak in this process was collected. The purity of CBD in this fraction was confirmed to be 71.5% using HPLC.

To produce pure CBD crystals from the CBD obtained through MPLC, we conducted crystallization, resulting in white powdered CBD. The purity of the powdered CBD was measured using HPLC, revealing a single peak at the same retention time as the standard substance, with a purity of 99.11% (Figure 4). These results confirmed that the material extracted and purified from hemp was highly pure CBD.

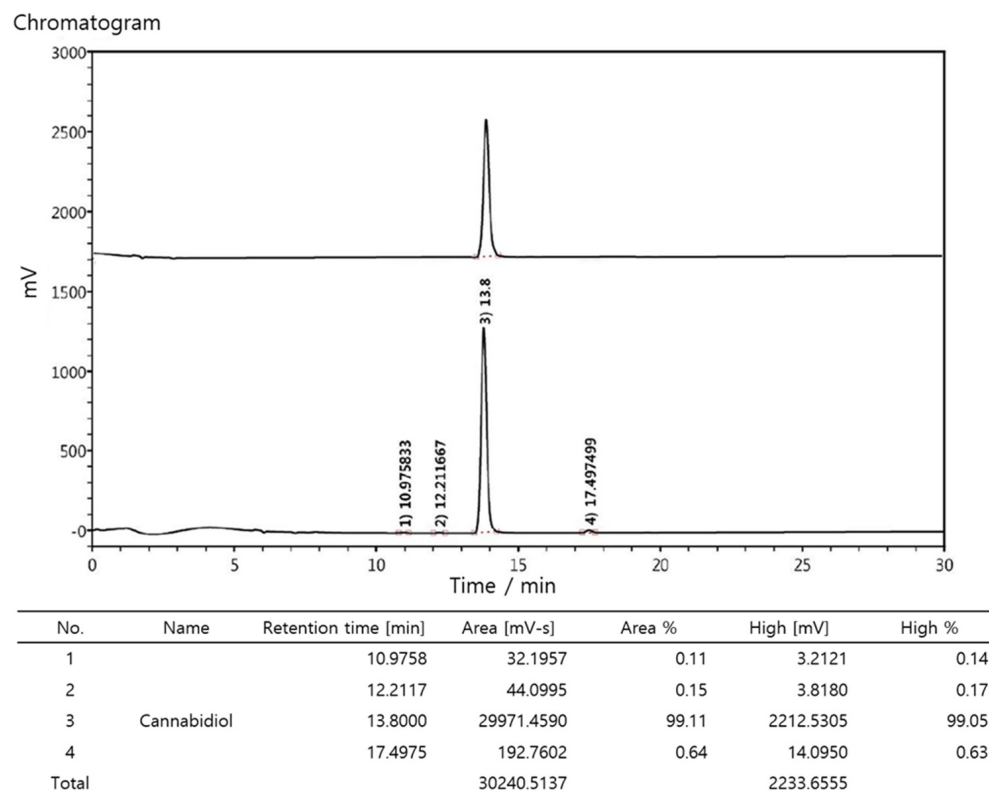


Figure 4. Chromatographic analysis for purity determination of cannabidiol (CBD) crystals.

4. Conclusions

In this study, we optimized the SFE conditions for CBD extraction from hemp using response surface methodology, with a temperature range of 35–70 °C, a pressure range of 20–50 MPa, and an extraction time of 60–120 min. The optimal conditions identified for extracting CBD were an extraction pressure of 48.3 MPa, a temperature of 60 °C, and an extraction time of 109.2 min, resulting in a CBD yield of approximately 70.46 g/kg. These findings suggest enhanced efficiency in industrial CBD extraction. Moreover, MPLC was employed to achieve a CBD purity of 99.11%, underscoring the effectiveness of these extraction conditions and the purification method.

The application of both the Box–Behnken design and the central composite design in RSM allowed for the precise optimization of the extraction parameters. The statistical analysis confirmed the significant impact of the independent variables on the CBD yield, ensuring the reliability and reproducibility of the optimized conditions. Response surface plots and contour plots provided valuable insights into the interaction effects of the variables, facilitating a deeper understanding of the extraction process.

Furthermore, the high-purity CBD obtained through MPLC was successfully converted from CBDA via heat treatment, demonstrating the effectiveness of the purification and conversion process. These optimized extraction and purification processes provide a robust framework for the industrial-scale production of high-purity CBD from hemp.

The findings from this study highlight the potential of SFE and RSM for the efficient, high-yield extraction of CBD from hemp. This approach not only enhances the industrial value of *Cannabis sativa* L. but also underscores its ecological and sustainable benefits as a crop with a high CBD content.

Further studies could focus on refining supercritical fluid extraction (SFE) conditions for the extraction of other valuable cannabinoids and terpenes, thus broadening the industrial application of this technique. Additionally, evaluating the sustainability of the SFE process, particularly its energy consumption and carbon footprint, could contribute

to its environmental optimization. Investigating the scalability of SFE for industrial-scale applications will also be critical for its wider adoption.

Moreover, combining SFE with other extraction technologies, such as solvent-based extraction or microwave-assisted extraction, could be a promising direction. This could improve both yield and efficiency, making the extraction process more effective for various compounds while enhancing its industrial viability.

To enhance the impact of this study on future research, several areas of exploration are suggested. First, while this study focused on optimizing CBD extraction, the optimized supercritical CO₂ conditions may be applicable to the extraction of other cannabinoids and bioactive compounds. These findings provide a foundation for further studies to explore various cannabinoids, such as THC and CBG, under similar optimized conditions. Additionally, combining supercritical CO₂ extraction with other green extraction methods—such as ultrasound-assisted or microwave-assisted extraction—presents an opportunity to enhance extraction yield and efficiency, potentially reducing the processing time and energy consumption. For industrial applications, the statistical models and parameters presented here serve as a robust starting point for large-scale extraction processes, supporting the more sustainable and high-yield production of hemp-derived products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr12112498/s1>, Table S1: Estimated regression coefficients for CBD; Table S2: Analysis of variance (ANOVA) for response of dependent variables; Table S3: Estimated regression coefficients for CBD Extraction Optimization; Table S4: Analysis of variance (ANOVA) for response of dependent variables CBD Extraction Optimization.

Author Contributions: M.H.: investigation, formal analysis, methodology, and writing—original draft; J.-H.K.: investigation, data curation, writing—original draft, and validation; C.-H.K.: funding acquisition and project administration; T.-H.K.: writing—review and editing, supervision, and conceptualization; and S.-U.L.: writing—review and editing, and supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Ministry of Science and the ICT (MSIT, 2021-DD-UP-0379) of the Republic of Korea.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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