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Supercritical CO$_2$ Extraction of Seed Oil from *Psophocarpus tetragonolobus* (L.) DC.: Optimization of Operating Conditions through Response Surface Methodology and Probabilistic Neural Network

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Abstract: For the treatment of menopausal symptoms, nutraceuticals and herbal remedies are thought to be more natural and safer than hormones. Attention has been paid to the winged bean (*Psophocarpus tetragonolobus* (L.)) DC. seed oil. They are constituted of phytosterols, which may be effective in preventing menopausal symptoms. The purpose was to determine the optimal conditions for supercritical fluid extraction of oleic-rich oil from winged bean seeds. To optimize the condition, the response surface methodology (RSM) and probabilistic neural network (PNN) were utilized. In this research, PNN was used to improve RSM estimation by reducing the number of calculations. The optimized extraction conditions for winged bean seed oil entailed a CO$_2$ flow rate of 21.3 L/h, a pressure of 30 MPa, a temperature of 55 °C, and an extraction time of 90 min. Under these conditions, the extraction process yielded a maximum oil yield of 36.27%. Ultimately, winged bean seed oil included a greater proportion of unsaturated fatty acids such as oleic acid, linoleic acid, and linoleic acid than oil produced using cold pressing or co-solvent extraction.

Keywords: *Psophocarpus tetragonolobus* (L.) DC.; supercritical fluid extraction; response surface methodology; probabilistic neural network; oleic acid

1. Introduction

Currently, the female population aged 40–59 years is the period of menopause. Menopause causes the function of sex hormones to decrease, which is expected to increase to 28% of the country's total female population in 2020 and has an increasing trend [1]. On average, women will reach menopause age around 50 years, while average life expectancy increases to 75 years.

Premenopausal and postmenopausal women (ages 40 to 59) are connected with alterations in a number of physiological activities, including a decline in sex hormones. Among the menopausal symptoms are urogenital atrophy, osteoporosis, and atherosclerosis. Nutraceuticals and herbal medicines are considered more natural and safer than hormones for the treatment of menopausal symptoms [2].

Thus, women will experience menopause for 25 years, or for one-third of their lifetimes. Even though this group of women is going through menopause, they play a significant role in the quantity and quality of national development. As a result, health promotion efforts are necessary. This is because women of this age have changed physically, intellectually, and emotionally because of estrogen decline. Menopause brings on hot flashes, muscle and joint pain, exhaustion, irritability, palpitations, sleeplessness, and urinary tract problems,

and includes problems that may not manifest early on, such as osteoporosis, head cramps, and vaginal dryness [1]. Age-related irregularities in women may also be mitigated by using natural health supplements. Menopause independent of hormone replacement oil derived from the seeds of the winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is being examined as a non-hormonal alternative to treat menopausal symptoms. According to research, the oil extracted from winged bean seed includes large quantities of phytosterols, including sitosterol, stigmasterol, and campesterol, second only to soy, which contain estrogen-like sex hormones [2].

Winged bean (*Psophocarpus tetragonolobus*) seed oil, rich in phytosterols, particularly sitosterol, stigmasterol, and campesterol, has shown potential in preventing menopausal symptoms. The study conducted by Kupittayanun et al. [2] investigated the protective effects of winged bean seed oil against sex hormone insufficiency using an animal model.

The results indicated that winged bean seed oil exhibited beneficial effects on various aspects related to menopause. It increased relative uterine weight, promoted vaginal cornification, and enhanced uterine endometrial proliferation, suggesting a positive influence on reproductive organs. The oil also showed potential in improving the interlobular ducts of the mammary gland, indicating a possible role in maintaining mammary health.

Furthermore, winged bean seed oil demonstrated a positive impact on lipid metabolism, reducing total cholesterol, triglyceride, and LDL cholesterol levels while increasing HDL cholesterol. This lipid profile improvement may contribute to cardiovascular health during menopause. Importantly, winged bean seed oil showed promise in preventing bone loss associated with menopause. Ovariectomized rats treated with the oil exhibited recovery in bone mineral density compared to the control group, suggesting a potential preventive effect against osteoporosis in postmenopausal women [2].

There are many ways to extract oil, including solvent extraction and cold compression, among others [3–6]. In recent years, research has been conducted on supercritical fluid extraction (SFE), which has numerous advantages over conventional techniques. The chemical reagent is not required as a solvent in the SFE procedure. It removes chemical impurities from the oil, making it environmentally and consumer friendly. Thus, it appears vital to use the widely used response surface methodology (RSM), a mathematical modeling technique, for the optimization assessment of the extraction process. This technique involves sophisticated calculations for the optimization process and the development of an acceptable experimental design that incorporates all the independent variables and uses the experimental data input to derive a set of equations that can yield a hypothetical value for output.

Supercritical fluid extraction (SFE) has emerged as a sustainable and efficient method for extracting seed oils in various industries, including food, pharmaceutical, and cosmetics. The unique advantages of SFE, such as selectivity, low extraction temperatures, and shorter processing times, make it an attractive choice for obtaining high-quality seed oils. This review has provided a comprehensive overview of the use of SFE in seed oil extraction, covering important aspects including extraction mechanisms, key parameters, process optimization through response surface methodology (RSM), and the application of semi-empirical models to understand thermodynamics and mass transfer processes. The findings highlight that SFE enables high extraction efficiency, cost-effectiveness, and time efficiency, particularly when incorporating modeling and optimization approaches. Furthermore, the combination of SFE with other extraction methods, such as ultrasound-assisted extraction, shows promise for further enhancing the extraction process. Overall, this review underscores the significance of SFE as a valuable technique for seed oil extraction, offering insights that can benefit diverse industries seeking sustainable and efficient extraction methods [7].

The primary objective of the study by [8] was to investigate the extraction of Passiflora seed oil using supercritical carbon dioxide (SC-CO_2) and to develop models for predicting the oil extraction yield. Artificial neural networks (ANN) and response surface methodology (RSM) were employed as modeling techniques to achieve this goal. Furthermore,

process optimization was conducted using both ANN and RSM to determine the optimal operating conditions that would yield the maximum extraction yield of Passiflora seed oil. The ANN model estimated the maximum extraction yield to be 26.55%, with a temperature of 56.5 °C, pressure of 23.3 MPa, and extraction time of 3.72 h. In comparison, the RSM model predicted an optimum oil extraction yield of 25.76% at a temperature of 55.9 °C, pressure of 25.8 MPa, and extraction time of 3.95 h. To assess the accuracy of the models, mean squared error (MSE) and relative error methods were utilized to compare the predicted values with the experimental data. The results of the comparison indicated the superior performance of the ANN model over the RSM model. These findings underscore the effectiveness of the ANN model in predicting and optimizing the extraction process for Passiflora seed oil using $SC-CO_2$ [8].

The study compared the quality of chia seed (*Salvia hispanica* L.) oil obtained through supercritical carbon dioxide ($SC-CO_2$) extraction with that of Soxhlet extraction and commercial cold-pressed oil. The effects of different extraction parameters (pressure, temperature, and grinding time of chia seed) on the yield of chia seed oil were investigated using response surface methodology (RSM). The results demonstrated that the linear and quadratic terms of pressure, temperature, and particle size (represented by grinding time) significantly influenced the amount of chia seed oil obtained. This study suggests that $SC-CO_2$ extraction is an effective method for producing chia seed oil with excellent sources of PUFAs and tocopherols. While the oil yield may be lower compared to Soxhlet extraction, chia seed oil obtained through $SC-CO_2$ extraction under optimized conditions exhibited comparable quality attributes to commercial chia seed oil. However, it is important to consider the higher susceptibility of chia seed oil to oxidation due to its elevated PUFA content [9].

The utilization of CO_2-expanded ethanol (CXE) as a green solvent for extracting oil from grape seeds showed promising results in terms of oil yield, extraction efficiency, and quality of the extracted oil. The optimized CXE extraction conditions resulted in a high oil yield of 13.6%, which was equivalent to or even higher than conventional methods. Additionally, the CXE-based process demonstrated advantages in reducing solvent consumption and extraction time. The extracted oil exhibited a significant proportion of unsaturated fatty acids, particularly linoleic acid, known for its high antioxidant capacity. Overall, CXE extraction offers a more efficient, environmentally friendly, and potentially superior alternative for oil extraction from grape seeds, with potential applications in the grape-processing industry [10].

A study successfully employed sequential extraction kinetic modeling and artificial neural networks (ANN) to optimize the supercritical fluid extraction (SFE) of raspberry seed oil. The mass transfer model proposed by Sovová provided the best fit for the experimental data, indicating its suitability for describing the extraction kinetics. The findings suggest that SFE should be performed at higher pressure and CO_2 flow rate, while maintaining a lower temperature and particle size, with the aim of achieving a maximal initial mass transfer rate. These insights contribute to the effective extraction of raspberry seed oil using SFE, offering potential applications in the food and pharmaceutical industries [11].

An artificial neural network (ANN) is an interconnected group of artificial neurons simulating the thinking process of the human brain. One can consider an ANN as a "magical" black box trained to achieve expected intelligent processes, against the input and output information stream. Thus, there is no need for a specified algorithm on how to identify different plants. PNN is derived from the radial basis function (RBF) network, which is an ANN using RBF. RBF is a bell shape function that scales the variable nonlinearly. PNN is adopted because it has many advantages. Its training speed is many times faster than a BP network. PNN can approach a Bayes optimal result under certain easily met conditions. Additionally, it is robust to noise examples. We choose it also for its simple structure and training manner. The most important advantage of PNN is that training is easy and instantaneous. Weights are not "trained" but assigned. Existing weights will never be alternated but only new vectors are inserted into weight matrices when training, so, it

can be used in real time. Since the training and running procedure can be implemented by matrix manipulation, the speed of PNN is very fast. The network classifies an input vector into a specific class because that class has the maximum probability of being correct. In this paper, the PNN has three layers: the input layer, the radial basis layer, and the competitive layer. The radial basis layer evaluates vector distances between the input vector and row weight vectors in the weight matrix. These distances are scaled by the radial basis function nonlinearly. Then, the competitive layer finds the shortest distance among them, and thus finds the training pattern closest to the input pattern based on their distance [12].

The probabilistic neural network (PNN) is a feed-forward neural network architecture that does not have cyclic connections between nodes. It serves as a classifier capable of approximating the probability density function for a given dataset. PNN is widely used in machine learning applications, particularly for classification and pattern recognition tasks. It offers an effective approach to address classification problems using a statistical memory-based strategy, whether supervised or unsupervised. By leveraging its ability to perform probabilistic inference, PNN enables accurate data analysis and categorization, leading to valuable insights and predictions. The probabilistic neural network (PNN) is a neural network model that incorporates principles from probability theory, such as Bayesian classification and alternative estimators for probability density functions. Its utilization of kernel functions enhances its effectiveness in discriminant analysis and pattern recognition tasks. The PNN offers a robust classification system by estimating probability density functions for different classes and utilizing Bayesian classification principles to assign new data points to the most likely class. The integration of kernel functions enables the PNN to identify and classify complex patterns in the input data. Overall, the PNN provides a powerful tool for various applications requiring accurate classification, pattern recognition, and discriminant analysis. One of the advantages of probabilistic neural networks (PNN) is their lack of dependence on back-propagation training, allowing for efficient and automated integration of new pattern units without incurring additional time costs. The basic architecture of the PNN is shown in Figure 1.
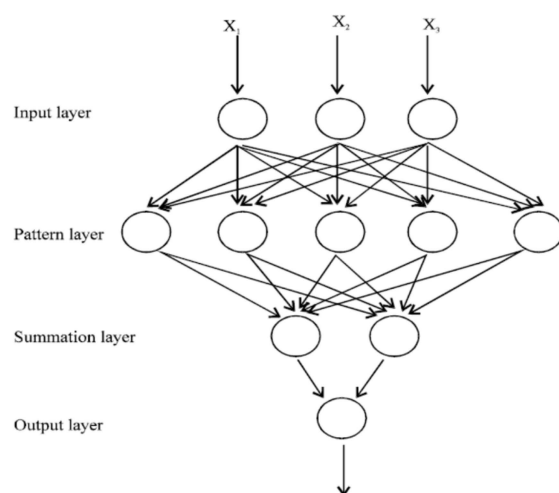
Figure 1. The basic architecture of the PNN adapted from [13].

For each dimension or feature in the training set, the easiest method is to use the standard deviation. Generalization is enhanced by using cross-validation (training datasets versus validation datasets). Cluster analysis is another approach. A training sample's neighborhood distances can be modeled [13], improved upon existing gap-based estimate methodologies. They estimated based on the number of features used and found that this was more reliable than estimates based on the number of classes. The estimation of σ in probabilistic neural networks (PNN) has been approached using genetic algorithms. Specifically, the PNN package in R language utilizes a genetic algorithm from the increased package to estimate σ. In a study by [14], three reinforcement learning techniques, namely Q (0)-learning, Q(λ)-learning, and

state-less Q-learning were investigated. The results obtained from these techniques demonstrated comparable performance to state-of-the-art approaches, showcasing their potential as viable alternatives in PNN modeling and analysis. Accordingly, the goal of this study is to compare RSM and PNN as a means of figuring out which method has a greater efficiency for optimizing extraction conditions and maximizing the production of winged bean seed oil under various pressure, time, and temperature circumstances. Furthermore, gas chromatography will be used to quantify the composition of the SFE-processed oil from both varieties of winged bean seed.

2. Materials and Methods

2.1. Sample Preparation

Winged bean seeds were obtained from Nakhon Ratchasima, Thailand. They were dried at 60 °C in tray dryer and finely ground to powder, kept in vacuum package at 4 °C until used.

2.2. Proximate Analysis

Dried winged bean seed powder was analyzed for ash, moisture, and fat by [15] as follows; ash (AOAC 900.02A), moisture (AOAC 925.10), and fat (AOAC 963.15).

2.3. SC-CO$_2$ Extraction

All SC-CO$_2$ extraction trials were conducted in a Speed® SFC supercritical fluid machine (Applied Separations, Inc., Hamilton Street, Allentown, PA, USA). Dried winged bean seed powder was weighed up precisely and put into the extraction container. Then, winged bean seed oil was extracted and weighed to obtain percentage yield of extraction.

2.4. Experimental Design

The experimental design employed in this study was the Box–Behnken design, which enables efficient exploration of the response surface with a limited number of experimental runs. The independent variables, namely extraction pressure (X1), extraction temperature (X2), and extraction time (X3), were set at various levels, including 20, 30, and 40 MPa for extraction pressure, 50, 55, and 60 °C for extraction temperature, and 60, 90, and 120 min for extraction time.

In addition, this study utilized response surface methodology (RSM) to optimize the extraction conditions using supercritical fluid. The experiments were conducted in a randomized order to ensure unbiased analysis. Regression analysis was performed to establish the relationship between the independent variables and the extract yield. Overall, the study aimed to identify the precise extraction conditions that would yield the highest extract yields.

2.5. Experimental Validation of the Optimized Condition

The yield response of extraction was optimized by being maximized conferring to the model. With the intention of validating the regression model created, three extraction tests were agreed with the optimal operating conditions, because they were diverse from those of the experimental used to develop the model.

2.6. Optimization Condition of Oil Extraction Using Probabilistic Neural Network Model

The PNN became interesting and useful in way of application for determination of the optimization of extraction conditions by obtaining more data points and obtaining more accurate extraction conditions and better statistical correlation results.

The probabilistic neural networks (PNN) and general regression neural networks (GRNN) are specialized neural network models used for classification and regression tasks, respectively. PNNs employ radial basis functions (RBF) and optimal sigma values to assign weights to data points, while GRNNs focus on regression problems with continuous target variables. PNNs draw conceptual similarities to K-nearest neighbor (k-NN) models but

extend the approach to consider all data points. The decision layer of PNN networks compares weighted votes from the pattern layer to predict the target category. DTREG, a data mining research tool, aids in determining optimal sigma values. Overall, PNNs and GRNNs offer effective solutions for categorical classification and continuous regression tasks, respectively, showcasing the power of neural network models in data analysis and prediction [5].

Implementation

The probabilistic neural network model was implemented using a data mining tool (DTREG) [5]. The classification problem is optimized with 15 variables as input data with 15 treatments from Box–Behnken design. The DTREG measures the residual error of the model using the yield percentage values for each iteration. If the error does not improve consecutive iteration, DTREG assumes the yield percentage has converged to the optimal values, and it stops the conjugate gradient process [5]. Training and validation data interpretations are discussed in detail in results and discussion.

2.7. Fatty Acid Composition by GC Chromatography

Fatty acid methyl esters (FAMEs) were prepared through transmethylation using boron trifluoride in methanol. The analysis of FAMEs was carried out using an Agilent 7890a instrument equipped with a fused silica capillary column (SP2560, Supelco Inc., Bellefonte, PA, USA) measuring 100 m × 0.25 mm × 0.2 μm. A flame ionization detector (FID) was employed, with both the injector and detector temperatures set at 260 °C. The column temperature was initially set at 70 °C and then ramped up at a rate of 13 °C/min until reaching 175 °C, followed by a further increase at 4°C/min until reaching 240 °C. Helium was used as the carrier gas, flowing at a rate of 20 cm/min. An aliquot of 1 μL of FAME was injected with a split ratio of 1:30. The identification and percentages of fatty acids were made by comparing the relative retention times of FAME peaks from sample with standards (37 component FAME Mix, catalog No. 47885-U, Supelco, Bellefonte, PA, USA) [16].

3. Results

3.1. Proximate Analysis

Dried winged bean seed powder is composed of moisture 9.22%, ash 4.91%, protein 33.83%, fat 17.51%, crude fiber 12.23%, and carbohydrate 22.30%. The results revealed that the Thai winged bean has high amounts of fat, which is an interesting topic for future research.

3.2. Response Surface Methodology

The winged bean seed oil extraction was optimized using the RSM methodology previously applied by Liu et al. [6]. However, this winged bean seed oil differs considerably to its main components as unsaturated fatty acids. The experimental design, data analysis, and optimization procedures were performed utilizing Design-Expert® software (Version 8.0.7.1, Stat-Ease, Inc., Minneapolis, MN, USA). To investigate the correlation between independent variables, a one-way analysis of variance (ANOVA) was conducted. The analysis aimed to assess the level of linear correlation between the experimental data and the predicted data generated from the mathematical model. The results of this analysis are presented in Table 1 and visualized in Figure 2a–c, highlighting the observed correlation between the variables.
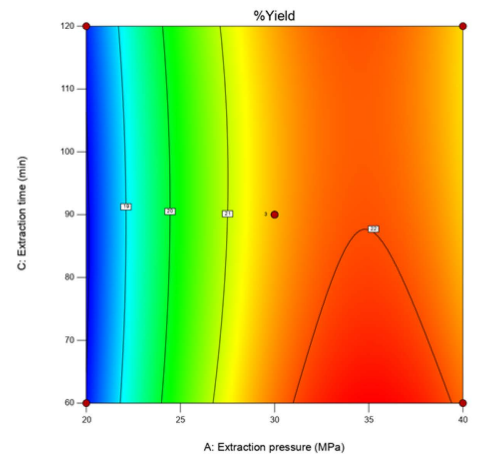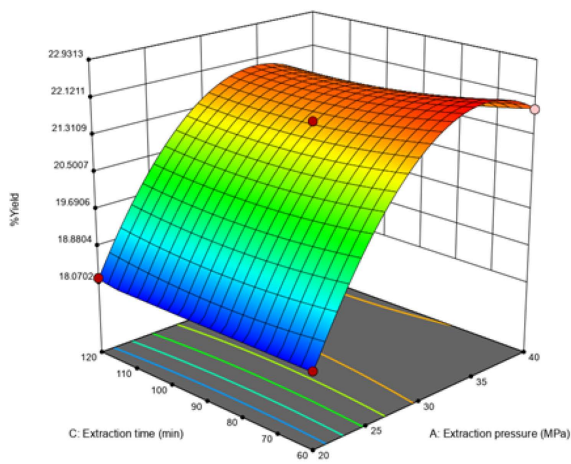
(**a**)
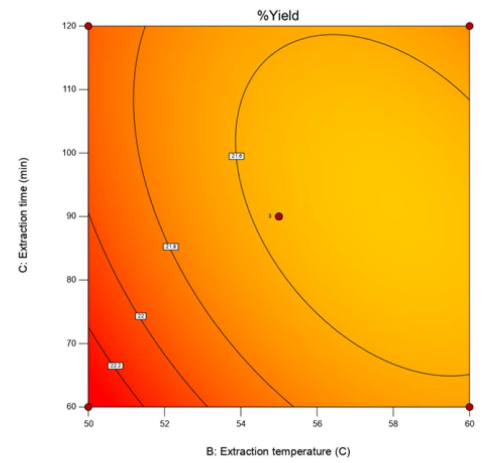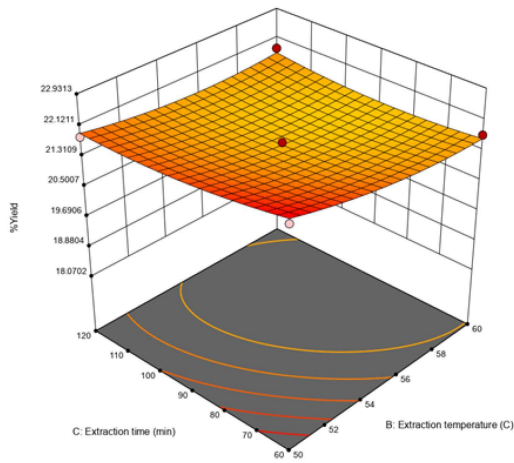
(**b**)
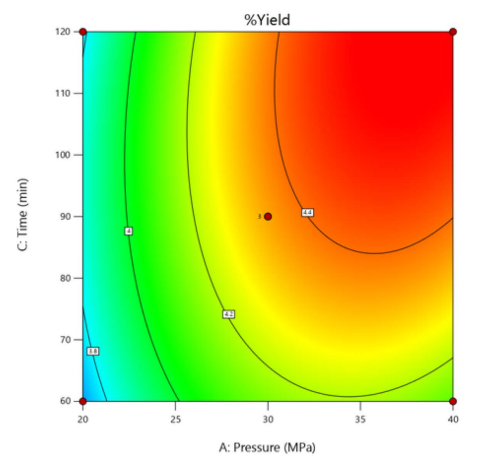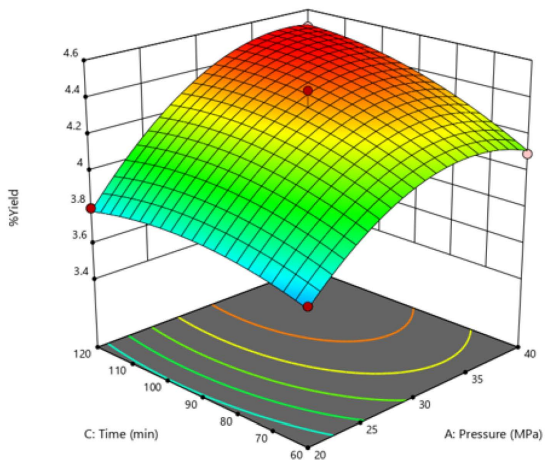
(**c**)

Figure 2. Response surface plots demonstrating the synergistic impact of extraction pressure and time on percentage yield of *Psophocarpus tetragonolobus* (L.) DC. oil at temperature 50 °C (**a**), pressure 30 MPa (**b**), and time 55 min (**c**).

Table 1. Experimental design and response of independent variables to the extract parameters for *Psophocarpus tetragonolobus* (L.) DC. seed.

Exp No. [a]	Independent Variables			*Psophocarpus tetragonolobus* (L.) DC.		
	Pressure (MPa) X_1	Temp. (°C) X_2	Time (min) X_3	%Yield (Y_1) Experimental	%Yield (Y_1) RSM Predicted	%Yield (Y_1) PNN Predicted
1	20	50	90	18.18 ± 0.08 [f]	18.11	18.18
2	20	55	60	18.09 ± 0.08 [f]	18.04	18.09
3	20	55	120	18.16 ± 0.31 [f]	18.14	18.16
4	20	60	90	18.00 ± 0.09 [f]	18.14	18.00
5	30	50	60	22.29 ± 0.23 [a]	22.41	22.29
6	30	50	120	22.84 ± 0.25 [b]	21.93	21.84
7	30	55	90	21.63 ± 0.35 [bcd]	21.56	21.43
8	30	55	90	21.43 ± 0.06 [cde]	21.56	21.43
9	30	55	90	21.62 ± 0.26 [bcd]	21.56	21.43
10	30	60	60	21.74 ± 0.07 [bc]	21.65	21.74
11	30	60	120	21.84 ± 0.07 [b]	21.72	21.84
12	40	50	90	22.33 ± 0.06 [a]	22.19	22.33
13	40	55	60	21.88 ± 0.06 [b]	21.90	21.88
14	40	55	120	21.35 ± 0.15 [de]	21.40	21.35
15	40	60	90	21.10 ± 0.37 [e]	21.17	21.10

[a] Experiments were conducted in a random order. All superscript small letters mean significant differences at *p*-value < 0.05 when compared in same column.

3.3. Fitting Model

The experimental data as the percentage yields (Y_1) of pomegranate oil achieved from all the experiments were applied to define the regression coefficients. The designated percentage yields created a significant model, confirming that at least one of the variables in extraction could clarify the variation of the response variable in association with its mean. The regression coefficients and regression model are specified in *Psophocarpus tetragonolobus* (L.) DC. seed oil equation obtained.

$$Y = 22.7375 + 1.62812\,X_1 - 0.854\,X_2 - 0.072292\,X_3 - 0.00525\,X_1X_2 - 0.0005\,X_1X_3 + 0.000917\,X_2X_3 - 0.018676\,X_1{}^2 + 0.008\,X_2{}^2 + 0.000188\,X_3{}^2; \text{quadratic polynomial}$$

(1)

The coefficient of multiple determination (R^2) demonstrated a substantial value of 0.9407, indicating a strong relationship between the model and the experimental results. Furthermore, the *p*-value for the lack of fit test corroborated the adequacy of the model in representing the experimental outcomes. These findings collectively support the notion that the model effectively captures and represents the observed experimental results.

3.4. Analysis of Regression Coefficients

A significant ($p < 0.0001$) positive linear of pressure was found for the highest percentage yield of oil extraction, while a tendency ($p < 0.05$) was observed for extraction time, indicating that an increase in extraction time increases the percentage yield of oil. A tendency ($p > 0.05$) towards negative quadratic temperature effects was observed for percentage yield implying that its extraction increases up to an optimal temperature after which it starts to decrease (Figure 2a). Three-dimensional plots could be used to demonstrate response surfaces by depicting the response as a function of two parameters while holding the third constant. Extraction pressure, temperature, and time were found to have a significant impact on the output of winged bean seed oil as Figure 2b,c. The response surface plot demonstrates the impact of both extraction time and temperature on the final product on the percentage yield of winged bean seed oil at an extraction pressure of 30 MPa (Figure 2b). The response surface schemes indicate the interaction effect of extraction pressure and extraction time on winged bean seed oil percentage yield by extraction temperature at 55 °C (Figure 2c).

3.5. Validation of the Model

The improved extraction method was selected by grouping extraction variables at the highest percentage yield (22.29 and 4.50%) for winged bean seed oil. Precisely, this agreed to the extraction pressure at 40 MPa, extraction temperature at 50 and 55 °C, and extraction time at 90 and 120 min for winged bean seed oil; three extractions were performed under those conditions to confirm the model's prediction (Table 2). No alterations were made between the predicted and experimental values of the percentage yield which established the model's accuracy. In addition, data were investigated for correlation among independent variables and percentage yield; statically analysis was significant at a *p* value less than 0.01.

Table 2. Analysis of variance of (ANOVA) independent variables for the extraction of oil from *Psophocarpus tetragonolobus* (L.) DC. Note R-square 0.9721, degree of freedom (DF).

Source	*Psophocarpus tetragonolobus* (L.) DC.					
	Sum of		Mean	F	*p*-Value	
	Squares	df	Square	Value	Prob > F	Significant
Model	39.80	9	4.42	172.09	<0.0001	significant
A-Pressure	25.31	1	25.31	985.08	<0.0001	
B-Temp	0.4802	1	0.4802	18.69	0.0075	
C-time	0.0820	1	0.0820	3.19	0.1341	
AB	0.2758	1	0.2758	10.73	0.0221	
AC	0.0900	1	0.0900	3.50	0.1202	
BC	0.0756	1	0.0756	2.94	0.1460	
A^2	12.74	1	12.74	496.80	<0.0001	
B^2	0.1477	1	0.1477	5.75	0.0618	
C^2	0.1038	1	0.1038	4.03	0.1000	
Residual	0.1285	5	0.0257			
Lack of Fit	0.1031	3	0.0344	2.71	0.2814	not significant
Pure Error	0.0254	2	0.0127			
Cor Total	39.92	14				

Std.Dev = 0.1603; R-Squared = 0.9968; Mean = 20.77; R-Squared = 0.9910; C.V.% = 0.7719; Adeq Precision = 33.3794.

3.6. Data Acquisition and Preprocessing for PNN

Practically, in a laboratory when we need to determine the optimization condition of extraction, it was time-consuming and quite complex to design an experiment for three varying parameters with each having three levels, so the application of the Box–Behnken design needed to be carried out to reduce the number of treatments. Yet still, we could obtain better optimized results from applying PNN when compared with RSM.

The utilization of the PNN model with Parzen probabilistic density function (pdf) estimators offers significant benefits in optimizing conditions. By approaching the underlying parent density, these estimators allow for the generation of a new dataset with increased data points, leading to a more accurate determination of the optimized conditions. The evaluation of the PNN model's performance through the comparison of actual and predicted values, as well as the graphical representation of the linear regression analysis. Additionally, when compared to RSM, as seen in Figure 3. the PNN offers insights about its predictive accuracy, as seen in Figure 4.

Overall, the application of the PNN model with Parzen pdf estimators proves to be a valuable approach for optimizing conditions. The combination of statistical methods and neural network techniques enhances the accuracy and reliability of predictions, facilitating informed decision-making in various applications.

The study by Wu et al. [4] highlights the successful implementation of the PNN algorithm for plant classification, providing an accurate and efficient artificial intelligence approach. The results emphasize the value of combining PNN with image and data processing techniques in developing automated systems for plant recognition.
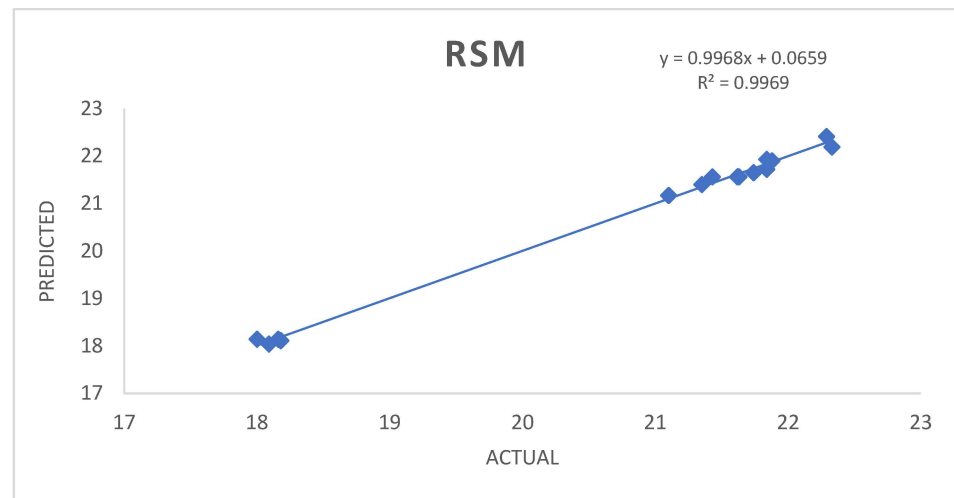
Figure 3. A plot of predicted and experimental value for the % yield of oil extracted from wing bean seed oil using RSM.
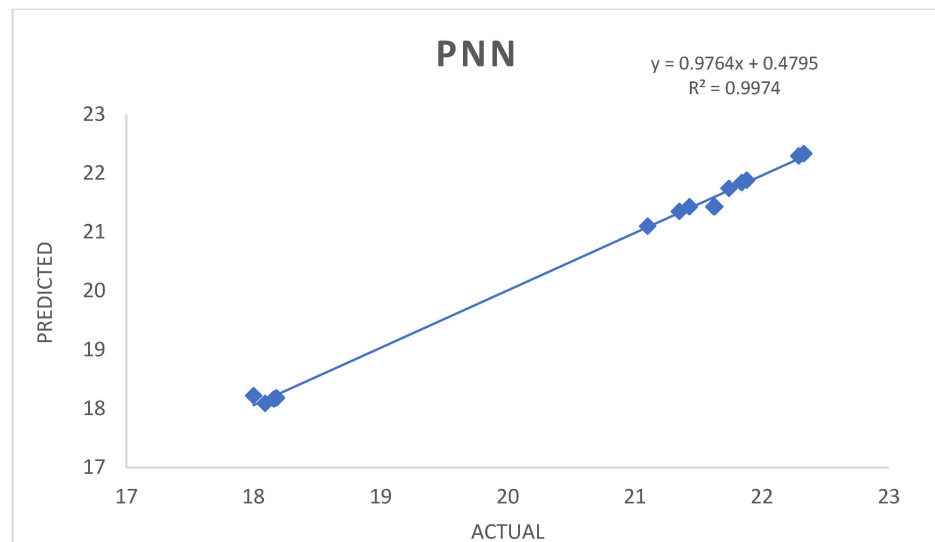

Figure 4. A plot of predicted and experimental value for the % yield of oil extracted from wing bean seed oil using PNN model.

3.7. Fatty Acid Composition by GC Chromatography

Data shows the GC chromatography of fatty acid in winged bean seed oil. The winged bean seed oil showed the presence of saturated fatty acids such as palmitic acid (C16:0) stearic acid (C18:0), arachidic acid (C20:0), and lignoceric acid (C24:0). In addition, both winged bean seed oils showed the presence of unsaturated fatty acids such as oleic acid (C18:1n9c), α-linoleic acid (C18:3n3), and γ-linoleic acid (C20:3n6). Furthermore, only winged bean oil presented eicosatetraenoic acid; EPA (C20:5n3). The fatty acid profile of winged bean seed oil is shown as detailed in Table 3 and Figure 5.

Table 3. Fatty acid profile of *Psophocarpus tetragonolobus* (L.) DC. oil prepared by SCF extraction.

Ret Time (min)	Type	Area (Pa·S)	Amt/Area	Norm (%)	Grp	Name
27.576	BB	1860.14294	9.55544×10^{-4}	8.904276	Palmitic acid sat	C16:0
34.353	BB	1119.09790	9.45590×10^{-4}	5.301195	Stearic acid sat	C18:0
37.310	BV	6894.15332	9.41893×10^{-4}	32.530017	Oleic acid w-9 FA	C18:1n9c
41.754	BB	5824.99316	9.88680×10^{-4}	28.850469	Linoleic acid	C18:2n6c
43.689	BB	357.63098	9.27769×10^{-4}	1.662175	Arachidic acid	C20:0
45.756	BV	616.17108	8.11596×10^{-4}	2.505202	Gondoic acid	C20:1
45.846	VB	326.53094	1.23414×10^{-4}	2.018783	α-Linolenic acid ALA	C18:3n3
51.089	BB	2508.13867	1.05127×10^{-4}	13.208935	Dihomo-γ-linolenic acid DaLA	C20:3n6
52.369	BB	128.73241	9.51279×10^{-4}	0.613476	Erucic acid	C22:1n9
56.525	BV	236.72110	1.31415×10^{-4}	1.558419	Eicosapen senoic acid	C20:5n3
57.607	BB	608.73352	9.33612×10^{-4}	2.847052	Lignoceric acid	C24:0
Total:				100.000000		
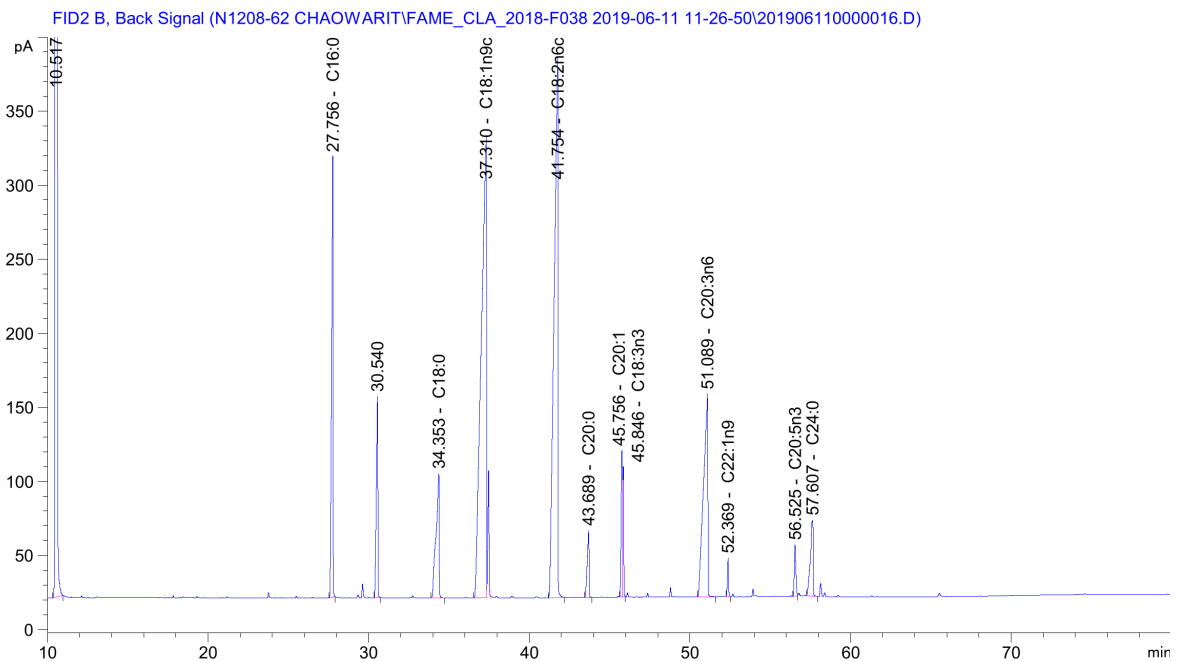
Figure 5. Gas chromatography of fatty acid from winged bean oil by SCF extraction method.

4. Discussion

The proximate analysis results of winged beans in this study are close to the report of the biochemical composition of winged beans by Bepary et al. [17]. Dried winged bean seed powder is composed of moisture 9.22%, ash 4.91%, protein 33.83%, fat 17.51%, crude fiber 12.23%, and carbohydrate 22.30%. The results revealed that the Thai winged bean has a high amount of fat, which is an interesting topic for future research.

The application of response surface methodology (RSM) for optimizing the extraction conditions revealed that the most favorable parameters for extracting winged bean seed oil using supercritical carbon dioxide (SC-CO$_2$) were a pressure of 30 MPa, a temperature of 55 °C, and an extraction time of 90 min. Under these optimal conditions, the extraction process resulted in a significant yield of approximately 34% for winged bean seed oil. Previous study successfully optimized the extraction of oil from Gliricidia sepium seeds using supercritical carbon dioxide (SC-CO$_2$) and response surface methodology (RSM). The results emphasized the significant impact of co-solvent addition on the oil yield during SC-CO$_2$ extraction. By employing RSM face-centered central composite design (FCCD), the study determined the optimal extraction conditions considering pressure, temperature, and CO$_2$ flow rate. The developed second-order polynomial model with an extended cubic

interaction demonstrated an excellent fit to the experimental data, with a high coefficient of determination (R2 = 0.98). The optimal conditions for achieving maximum oil yield (12.12%) were identified as 60 °C temperature, 40 MPa pressure, and 2.5 mL/min CO_2 flow rate, while keeping the extraction time constant at 60 min. These findings underscored the substantial influence of pressure, temperature, and CO_2 flow rate on the oil yield during SC-CO_2 extraction [18]. In addition, Liu et al. [19] studied pomegranate seed oil extraction using supercritical carbon dioxide. Response surface methodology was applied to assess the effects of the process parameters on the pomegranate seed oil percentage yield. The extraction parameters were enhanced with a central composite design experiment. All parameters and the interactions between pressure and temperature, as well as CO_2 flow rate and temperature, had significant effects on the oil yield ($p < 0.05$). The highest pomegranate seed oil percentage yield from the mathematical model was predicted to be 156.3 g/kg dry basis under the condition of pressure 37.9 MPa and temperature 47.0 °C with a CO_2 flow rate of 21.3 L/h.

Abbasi and Mahlooji [20] reported the use of response surface methodology (RSM) for exploring the relationships between explanatory variables and response variables. RSM aims to simplify complex real-world problems by employing polynomial estimation and optimization techniques such as the gradient method to obtain an optimal response. However, the limitations of polynomial estimation and the tendency to get trapped in local minima/maxima pose challenges for RSM. To address these limitations, the article proposes the use of neural networks within the RSM framework to enhance the estimation process and reduce calculations. Additionally, simulated annealing is suggested as a technique for maximizing the estimated objective function and reaching a suitable solution. Three examples with varying complexities are solved to demonstrate the effectiveness of the proposed method. The comparison results indicate that the proposed algorithm outperforms the classical method, highlighting its superiority in tackling complex problems and improving the accuracy of estimations within the RSM context.

In fatty acid, according to Kanth [21], the extracts obtained using organic solvent such as methanol–chloroform had higher concentrations of oleic acid, but the same concentration of linoleic acid extracted with pressurized CO_2 in this research. Moreover, the eicosapentaepoic acid was detected in this extraction method, which was detected before [21–23]. In addition, according to Lalas and Tsaknis [24], the extracts using organic solvents such as n-hexane, chloroform, and methanol, including cold press had related concentrations of MUFA and PUFA such as oleic acid, linoleic acid, and α-linoleic acid with pressurized CO_2 in this research. In addition, there is γ-linoleic acid present with pressurized CO_2 but not found with cold press and organic solvent extraction [25–27].

5. Conclusions

The optimum conditions to prepare oil from pomegranate seed with the highest percentage yield was determined using a randomized Box–Behnken design and data were used for statistical analysis by multiple regression. The condition that showed the highest percentage yield was optimized by RSM. The optimal condition for winged bean seed oil extraction by SC-CO_2 was at a pressure of 30 MPa, temperature of 55 °C, and extraction time of 90 min, which gave the highest yield at 36.27%. The implementation of the statistical regression model, following the proposed research design, demonstrates a clear positive correlation between the experimental data and the predicted data obtained from the probabilistic neural network (PNN). This indicates a strong relationship and consistency between the observed experimental outcomes and the predictions generated by the PNN model. Finally, the fatty acid profile and bioactive compounds in winged bean seed oil were determined.

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