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Investigation on Supercritical CO_2 Extraction of Black Nightshade Berries (*Solanum nigrum* Linn.)

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Abstract: A set of supercritical extractions (SCE) using carbon dioxide (CO_2) has been performed on *Solanum nigrum* Linn. A design of experiment (DoE) using Box-Behnken was applied to investigate the influential parameters on SCE. The relationship between extraction parameters (i.e., temperature, time, and pressure) and extraction products (i.e., phenolics-TPC, alkaloids-TAC, and flavonoids-TFC) was evaluated. It was found that temperature and pressure are the most influencing parameters in the SCE. Both give a synergistic effect in increasing the extraction yield. The optimum SCE conditions are 333 K, 30 min, and 240 bar; with extraction, yields expressed as TPC, TFC, and TAC are 55.1677 mg GAE/g extract, 28.0308 mg QE/g extract, and 5.9460 mg HYE/g extract, respectively. Solubility data correlations were done by using Chrastil's model. The adjustable parameters of Chrastil's model were found to be consistent with their physical meaning and can be applied in the SCE of *Solanum nigrum* Linn.

Keywords: *Solanum nigrum*; box behnken; supercritical extraction; chrastil; optimization.

1. Introduction

Solanum nigrum Linn. (SNL) is a bushy tropical-weed plant with berries, native from Eurasia. SNL berries are potentially toxic, especially when unripe; the risk of poisoning causes SNL utilization to be sparse. Aside from their toxicity, proper treatment of the mature SNL berries can provide beneficial therapeutic effects. This is since SNL berries contain bioactive compounds that show anti-diabetic, anti-oxidant, anti-tumor, anti-cancer, and anti-inflammatory [1-3]. Several studies have been conducted to examine the biological activity of the SNL plant extracts. Chen and co-workers showed that acetic acid-methanol extract of SNL has parasitemia suppression activity [4]. Some studies reported that SNL extract possesses a cytotoxic activity to several cell lines such as HL-60, Jurkat, Hep3B, and HepJ5.3 [5, 6]. The investigation by Patel *et al*. (2014) showed that chloroform extract of SNL gives active compounds that can inhibit mucositis [5]. Cyclohexane-methanol extract of SNL contains steroidal saponins with anti-inflammatory activity [6]. Furthermore, aqueous extracts of SNL show anti-diabetic activity [7].

The application of green extraction using supercritical fluids for extracting the pharmacologically active compounds from SNL has not been documented to date. Supercritical extraction (SCE) is considered more environmentally friendly than other extraction methods, e.g., solvent-mediated extraction, microwave-assisted extraction (MAE), and sonication-assisted extraction (SAE) [8-10]. The conventional solvent-mediated extraction is indeed unsuitable for the extraction of phytochemicals due to the inefficient and high risk of thermal degradation. Also, using a large volume of organic solvents raises pollution problems in the surrounding environment. It requires costly post-extraction stages to obtain high purity and safe-consumed products. SAE and MAE techniques are both superior to the conventional solvent-mediated method in terms of extraction period and performance, in addition to lower risk of thermal degradation [11-13]. However, the use of organic solvent and its following purification stages can introduce a high level of contamination into the product, which can be detrimental for biological applications.

SCE has gained increasing attention and is an up-and-coming technique for isolating various active phytochemicals from the plant matrices. The merits of SCE (which make this method expeditious and straightforward) emerge from its short extraction time, high precision, and flexible application due to the possibility of continuous modulation of the solvent power by manipulating pressure and temperature [11, 12, 14-17]. Further advantage coming from the recyclability of the extraction solvent. Thus it can significantly reduce the operating costs and minimize waste generation. Among the supercritical solvents, carbon dioxide (CO_2) is the most notable owing to its moderate critical temperature and pressure (304.25 K and 7.38 MPa, respectively), very low toxicity, safe handling, and storage, inexpensive, and easily obtained in ultra-high purity [18-20].

The present study aims to optimize the SCE using CO_2 as a solvent towards pharmacologically active compounds existing in SNL berries. There is no study dealing with the SCE of active compounds in SNL to the best of our knowledge. Parameter optimization and correlation were conducted using the response surface method —Box-Behnken design (BBD). The SCE parameters including temperature, pressure, and time were optimized towards the total alkaloid content (TPC), total flavonoid content (TFC), and total phenolic content (TPC) of the resulting extracts. The amount of alkaloid and phenolic compounds dissolved in CO_2 can be assumed as the solubility of natural active compounds extracted by supercritical CO_2. This information is critical for designing and optimizing scale-up extraction processes. The equilibrium solubility data was modeled using Chrastil density-based equations.

2. Materials and Methods

2.1. Materials.

The whole plant of SNL was purchased from a distribution agent of PT. Supra Boga Lestari, Surabaya, East Java, Indonesia. The SNL was harvested from the plantation in Pangalengan (7.1989° S, 107.5505° E), West Java. Ultra-high-purity grade CO_2 (99.995% purity) was purchased from PT Aneka Gas Industri, Sidoarjo, East Java, Indonesia. Absolute ethanol (99.8%) and Folin-Ciocalteu (F-C) reagent were purchased from Merck (Darmstadt, Germany). Standard gallic acid (98%), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Bromo cresol Green (dye content 95%), L-hyoscyamine (98%), vitexin (95%), quercetin dihydrate (98%), and potassium acetate (98%) were obtained from Sigma-Aldrich, Singapore. All the chemicals were used as received without further purification. All solutions were prepared with distilled

water obtained from a Millipore Milli-Q water purification system (specific resistivity of 18.2 MΩ·cm).

2.2. Supercritical extraction (SCE) of SNL berries.

Before the extraction, the SNL berries were removed from the stem and repeatedly washed with tap water to remove surface dirt and dried in an oven at 40 °C for at least 48 h until the moisture content was reduced to 6.5%. The dried SNL was then crushed and sieved using a Retsch AS 200 sieve shaker (Retsch GmbH) and the fraction retained on the +/- 70 mesh sieve was collected and weighed. Finally, the SNL powder was stored in an airtight plastic container before SCE experiments.

The SCE of dried SNL berries was carried out using a laboratory-scale supercritical fluid extraction apparatus, which comprised of a high-pressure metering pump (Eldex AA-100-S-2-CE, USA) that can dispense flow rates between 0.2 and 10 mL/min, a pressure transducer (Druck PTX-611, 0.1-700 bar), a temperature-controlled oven (Memmert UNB 500) for heating the system, and a stainless steel high-pressure reactor with an internal volume of ~150 cm^3. The maximum operating pressure, temperature, and flow rate of the extraction system were 400 bar, 300 °C, and 10 mL/min, respectively. Briefly, 20 g of SNL powder was placed in the extraction vessel, and cotton wool was packed at both ends of the cell to prevent solid particles' carryover into the tubing and clogging the system. The extraction cell was then placed in the heating chamber and heated to the desired temperature. Subsequently, liquid CO_2 was pumped into the system by a high-pressure metering pump at a rate of 10 cm^3/min until the target pressure was reached.

The supercritical extraction was performed under varied pressures of 80-240 bar and temperatures of 40-60 °C. After the desired conditions were achieved, the static extraction (CO_2 flow stopped) was started until the equilibrium condition of extraction was met (2h). Separation of the bioactive compounds from CO_2 was performed by using liquid solvent trapping. In such a trapping system, the extracts were recovered by bubbling CO_2 into a collection flask containing 10 mL of 99.8% v/v ethanol at ambient temperature. A trap was used to prevent any losses with the expanded CO_2, whose flow was measured by a wet gas meter. During the decompression of CO_2, the transfer tube was gently heated to prevent the non-volatile analytes' precipitation. Ethanol was used to collect the bioactive extract. The bioactive extract-containing ethanol was kept in amber bottles and stored at 4°C until further analysis. All experiments were carried out at least duplicated, and the average values of the yield and measurements were reported.

2.3. Characterization of the extracts by HPLC.

High-performance liquid chromatography (HPLC) analysis was conducted to determine the phenolic, flavonoid, and alkaloid compounds present in SNL extracts. The HPLC system consisted of a JASCO chromatograph equipped with a quaternary gradient pump (PU-2089 Plus), a JASCO type UV-2077 Plus variable-wavelength UV-Vis detector and an LC-NetII/ADC hardware interface. The separation was achieved on an Enduro C18 column (250 mm×4.6 mm, 5 μm) thermostated at 30 °C with mobile phases consisting of solvent A (water/acetic acid, 97:3, v/v) and solvent B (acetonitrile/acetic acid, 97:3, v/v). The injection volume was 5 μL with the mobile phase flow rate of 1 mL/min.

The following gradient elution program was employed to separate the major components of bioactive compounds from the column: 0 min 90% solvent A; 0 to 15 min solvent A from 90 to 80%; 15 to 38 min solvent A from 80 to 68%; 38 to 50 min solvent A from 68 to 54%; 50 to 55 min solvent A from 54 to 45%; 55 to 60 min solvent A from 45% to 0; and 60 to 70 min solvent A from 0 to 90%. Before each injection, the chromatographic system was equilibrated for at least 15 min. Identification of chromatographic peaks corresponding to bioactive compounds in SNL extracts was conducted by comparing the retention times and the UV spectra of the peaks in the samples' chromatograms with those of standards. Data processing was made using the ChromNAV software package (Version 1.18.04, Jasco Corp., Tokyo, Japan).

2.4. Determination of total phenolic content (TPC).

The TPC of SNL extract was determined using the Folin-Ciocalteu (F-C) assay following the procedure described by Habila *et al*. (2010) and Cavalcanti *et al*. (2012) with a slight modification [12, 21, 22]. Briefly, 2 mL of the diluted Folin-Ciocalteu reagent (1:10; v/v) was added to 1 mL of SNL extracts and incubated for 5 min at room temperature. Subsequently, 2 mL of Na2CO3 solution (7.5%, w/v) solution was added, and the mixture was kept in the dark for 60 min at room temperature. The absorbance at 765 nm was measured on a Shimadzu UV-1700 PharmaSpec UV-Vis spectrophotometer (Shimadzu Corp., Japan) against a blank containing distilled water instead of sample extract. Gallic acid was used as a standard for the determination of TPC. The results are expressed as gallic acid equivalents (GAE) using a standard gallic acid curve prepared with a series of gallic acid (0, 5, 10, 20, 40, 60, 80, and 100 µg/mL). The measurements were performed in triplicate, and the mean values were reported. The TPC (mg GAE/g SNL) was calculated according to equation (1):

$$\text{TPC} = \frac{\textbf{Read}\left(\frac{\textbf{mg}}{\textbf{mL}}\right)\times\textbf{100}}{\textbf{Sample weight (g)}} \quad (1)$$

2.5. Determination of total flavonoid content (TFC).

The TFC was determined by the aluminum chloride colorimetric method, as previously described by Woisky and Salatino (1998) with slight modifications [23-25]. Briefly, an aliquot of 0.5 mL of SNL extract was mixed with 0.1 mL of 10% (w/v) aluminum chloride, 0.1 mL of potassium acetate (0.1 M), and 2.8 mL of distilled water. The reaction mixture was then allowed to stand at room temperature for 30 min. After 30 min, the reaction mixture's absorbance was recorded at 415 nm using a UV-Vis spectrophotometer. The TFC was calculated from a calibration curve of quercetin (0-100 µg/mL). The results are expressed as milligram of quercetin equivalent per gram dry weight of SNL (mg QE/g SNL).

2.6. Determination of total alkaloid content (TAC).

The TAC of SNL extract was determined by a titrimetric method based on the complex formation between alkaloid and bromocresol green. In a typical procedure, one mL of SNL extract was dissolved in 2.0 mL acetone and 10.0 mL ether. The mixtures were transferred to a 250 mL volumetric flask, followed by the addition of 5 mL bromocresol green solution. The bromocresol green solution was prepared following the procedure described by *et al*. The mixture was then titrated with 0.005 N H_2SO_4 until the appearance of light green color (endpoint). Each mL of acid used is equivalent to 7.25 mg of alkaloid calculated as

hyoscyamine equivalent (HYE). The percent total alkaloid was calculated by the following equation:

$$\% \text{ Alkaloid} = \frac{\textbf{Weight of Alkaloid}}{\textbf{The weight of the plant sample}} \times 100\% \quad (2)$$

3. Results and Discussion

3.1. Experimental design in SCE of SNL berries.

Major bioactive compounds, specifically phenolic, flavonoid, and alkaloid, were identified from the crude extract of SNL by using the HPLC chromatography analysis and were quantified as TPC, TFC, and TAC, respectively. The response surface methodology (RSM) involves the statistical design of experiments where all factors are varied together over a set of experimental runs, which can be used to develop, improve, and optimize processes [26-30]. This technique has been applied in many areas of applied physics and engineering to reduce the cost of expensive analysis methods and their associated numerical noise. A computer software, namely Minitab 19.2. (Minitab Inc., PA, USA), was used to design the experimental matrix and to perform the necessary statistical analyses. For the experimental design, a three-variable three-level Box-Behnken design (BBD) was applied to optimize the extraction conditions for obtaining high recovery of bioactive compounds from SNL powder. The factors considered for experimental design are the extraction temperature (K, X_1), extraction time (min, X_2), and pressure (bar, X_3), where each factor was set at three levels, thus generating a total of 15 experimental runs as shown in Table 1.

Table 1. Experimental design (coded and uncoded levels) and results of the response variable.

No.	X_1	X_2	X_3	TPC[a]	TFC[b]	TAC [c]
1	313	30	240	9.84	7.10	2.44
2	313	30	80	2.95	1.37	0.55
3	313	75	160	7.95	5.25	1.55
4	313	120	240	8.98	6.38	2.08
5	313	120	80	2.05	1.45	0.59
6	323	30	160	17.78	8.58	2.65
7	323	75	240	25.8	13.08	3.67
8	323	75	160	17.15	8.62	2.73
9	323	75	80	3.17	1.42	0.59
10	323	120	160	17.6	8.69	2.77
11	333	30	240	59.01	30.21	6.30
12	333	30	80	7.78	2.28	0.97
13	333	75	160	7.07	2.09	0.92
14	333	120	240	57.95	29.57	6.15
15	333	120	80	6.95	2.14	0.96

[a] Phenolics extract expressed as mg gallic acid equivalent/g extract
[b] Flavonoids extract expressed as mg quercetin equivalent/g extract
[c] Alkaloids extract expressed as mg hyoscyamine equivalent/g extract

Extraction time, X_2, is an essential factor in the economic issue and risk of product degradation. Ye and Lai (2012), in the optimization of SCE conditions of onion oil using RSM, found the linear and quadratic terms of extraction time significantly affected the oil recovery [31]. In this study, a second-order polynomial regression model was developed to express the relationship between the response and the coded independent variables as follows:

$$Y = k_o + \sum_{i=1}^{n} k_i X_i + \sum_{i=1}^{n} k_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} k_{ij} X_i X_j \quad (3)$$

where Y denotes the predicted response of the process, which in this case corresponds to TPC, TFC, and TAC, X_i refers to the coded levels of the factors (or independent variables), and k_0, k_i, k_{ii}, and k_{ij} are the constant, linear, quadratic, and interaction coefficients, respectively. The coded and uncoded values of the independent variables used in the RSM design are presented in Table 1. The experimental sequence was randomized to minimize the effects of the uncontrolled factors.

The analysis of variance (ANOVA) was further employed to evaluate the credibility of the model response and the contribution of the linear, quadratic, and interaction terms to the response variable at the 5% significance level. The results are presented in Table 2. The response surface model's positive and negative coefficient values indicate synergistic and antagonistic effects between the corresponding independent variable and the response, respectively. From Table 2, the particular effects and interaction effect of pressure with temperature were highly significant in the case of TPC, TFC, and TAC of SNL extract, as indicated from P-values less than < 0.05. The small P-value (<0.05) indicates the more significant effect on the several response variables. On the other hand, the individual variables and their interaction forms gave insignificant effects on the yield of TPC, TFC, and TAC (P-value > 0.05). By neglecting the coefficients of the non-significant (P > 0.05) terms, the model equations for Y_{TPC}, Y_{TFC}, and Y_{TAC} can be expressed as follows:

$$Y_{TPC} = -3155 + 20.8X_1 - 0.50X_2 - 4.47X_3 - 0.0339X_1^2 + 0.00335X_2^2 + 0.000560X_3^2 - 0.00004X_1X_2 + 0.01381X_1X_3 - 0.000007X_2X_3 \quad (4)$$

$$Y_{TFC} = -1348 + 9.1X_1 - 0.024X_2 - 2.259X_3 - 0.0151X_1^2 + 0.00171X_2^2 + 0.000324X_3^2 - 0.00004X_1X_2 + 0.00698X_1X_3 - 0.000045X_2X_3 \quad (5)$$

$$Y_{TAC} = -762 + 4.50X_1 - 0.02X_2 + 0.325X_3 - 0.00655X_1^2 + 0.000449X_2^2 + 0.000037X_3^2 - 0.00016X_1X_2 - 0.001116X_1X_3 + 0.000022X_2X_3 \quad (6)$$

Table 2. Analysis of variance for the factors in the response surface model.

Source	DF	TPC		TFC		TAC	
		F	***P***	***F***	***P***	***F***	***P***
Model	9	6.53	0.03	5.53	0.04	6.34	0.03
Linear	3	14.14	0.01	11.98	0.01	15.17	0.01
X_1	1	15.82	0.01	8.95	0.03	8.42	0.03
X_2	1	0.02	0.89	0.01	0.93	0.02	0.90
X_3	1	26.58	0.01	26.99	0.01	37.08	0.01
Square	3	0.95	0.48	0.87	0.51	1.09	0.44
X_1^2	1	0.41	0.55	0.26	0.63	1.42	0.29
X_2^2	1	1.64	0.26	1.38	0.29	2.22	0.20
X_3^2	1	0.46	0.53	0.49	0.51	0.19	0.68
Two-way	3	4.50	0.07	3.73	0.10	2.75	0.15
$X_1 X_2$	1	0.00	0.10	0.00	0.99	0.00	0.95
$X_1 X_3$*	1	13.50	0.01	11.17	0.02	8.20	0.04
$X_2 X_3$	1	0.00	0.99	0.01	0.93	0.05	0.84

*The significant parameters in the SCE process of SNL

F and *P* represent the *F*-value and *P*-value, respectively

The R^2 values obtained for the model equations of Y_{TPC}, Y_{TFC}, and Y_{TAC} are 0.9216, 0.9087, and 0.9194, respectively. Furthermore, based on F-value and P-value (Table 2), one can see that the interaction between temperature (X_1) and pressure (X_3) has a synergistic effect, while the linear term of temperature, extraction time, and pressure all have antagonistic effects. Figure 1 depicts the optimum condition of each significant parameter in supercritical fluid extraction of TPC, TFC, and TAC from SNL. The optimum conditions for supercritical fluid extraction (within the SNL investigated conditions) are 333 K, 30 min, and 80 bar. The

optimum TPC, TFC, and TAC yields were 55.1677 mg GAE/g extract (0.93254 precision), 28.0308 mg QE/g extract (0.92444 precision), and 5.9460 mg HYE/g extract (0.93843 precision), respectively.

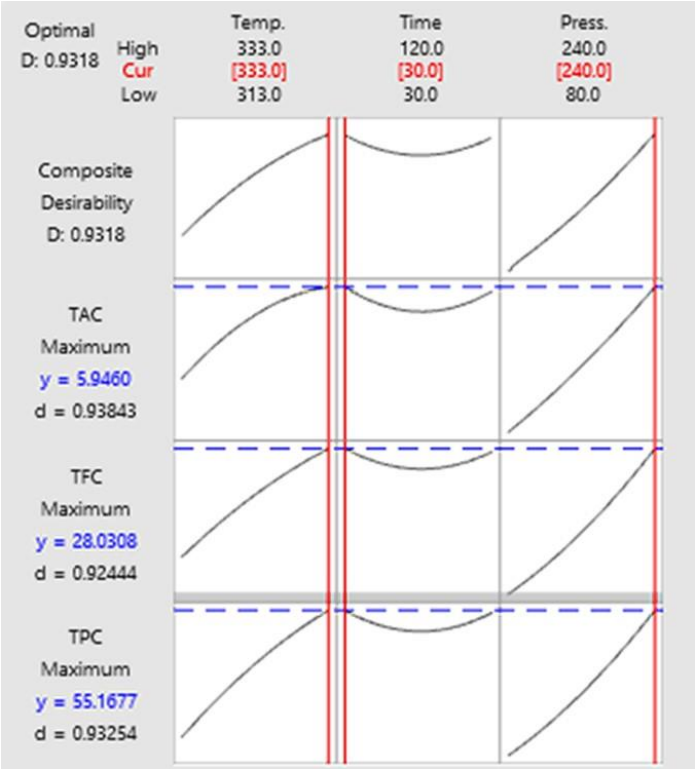

Figure 1. Optimization plot of extraction parameters of temperature (K), time (minute), and pressure (bar). The extraction yield and data precision are presented in y and d, respectively.

Primarily, temperature and pressure are the crucial factors influencing the yield of target compounds in supercritical fluid extraction, strongly related to the solvating power of fluids in the supercritical phase [32, 33]. In this regard, the pressure affected the density of CO_2, while temperature affected the vapor pressure and diffusivity of target compounds. Since the dissolving power of supercritical CO_2 is directly related to the CO_2 density, an increase in pressure at a constant temperature allows higher extraction efficiency of bioactive compounds as a result of enhanced. In contrast, the density of CO_2 decreased with temperature, which has a negative effect on the density of CO_2. The density and viscosity of CO_2 decreased with the increase of temperature. The decrease in viscosity with an increase in temperature significantly influences the mobility of CO_2 in the inner matrix of a solid; it will easily penetrate and reach the internal interior of the solid matrix. Therefore, a combination of pressure and temperature is the most important factor in the supercritical fluid extraction processes. The parameters that significantly affected the process also can be examined using the Pareto chart. The Pareto chart, Figure 2, shows the significance of the investigated extraction parameters to the yield of TPC, TFC, and TAC. It is identified that pressure and temperature are the influential parameters on TPC, TFC, and TAC yield, while extraction time was not a significant factor ($P > 0.05$). The increase of the yield with the rise in pressure is because of the enhanced density and solvent capacity of CO_2 as the extraction solvent.

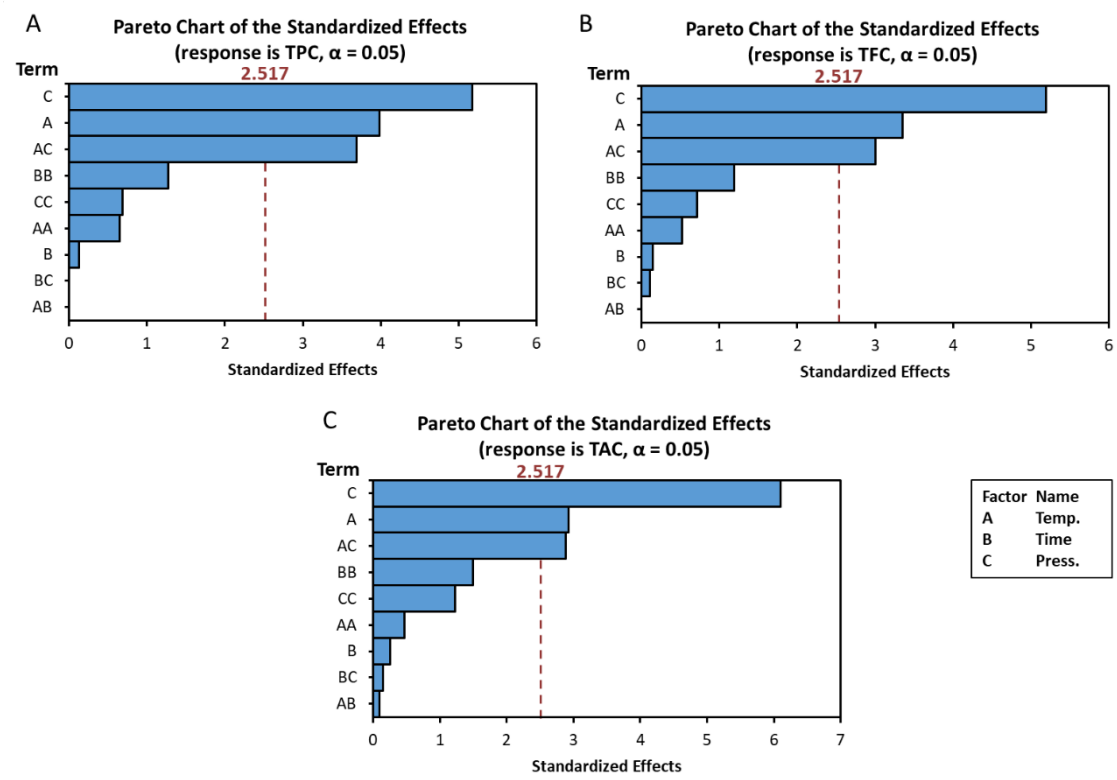

Figure 2. Pareto chart showing the effect of SCE parameters on the yield of TPC, TFC, and TAC.

The optimization study shows that temperature and pressure have a significant and synergistic effect in increasing the yield of SCE of SNL. The surface plot (Figure 3) shows that the increase of pressure and temperature supports the increase of TPC, TFC, and TAC yield. The enhancement effect can be correlated with an increase in the solute's volatility, which facilitates its dissolution [34, 35]. However, increasing temperature (only) while maintaining pressure at 80 bars does not significantly enhance the extraction yield.

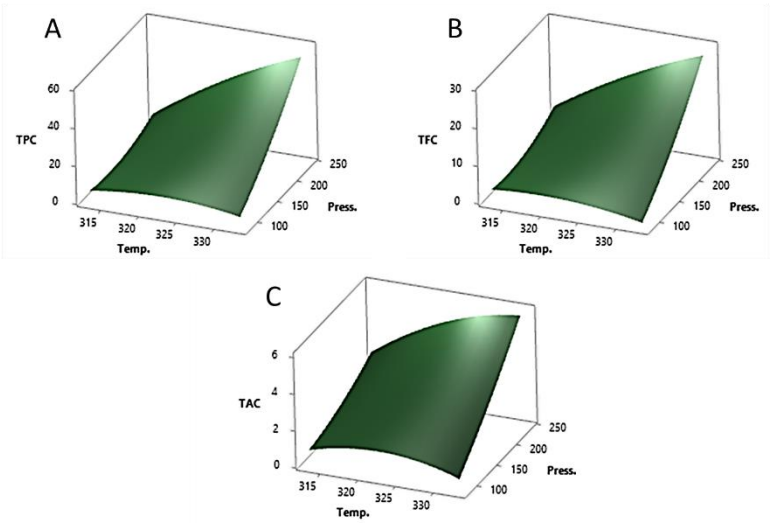

Figure 3. Surface plot showing the effect of SCE pressure and time on the yield of TPC, TFC, and TAC.

3.2. Correlation of the TPC, TFC, and TAC by density-based model.

As previously mentioned in the response surface method-BBD analysis, the combination of pressure and temperature was the most crucial factor in the SCE of bioactive compounds from SNL berries. The extraction efficiency in terms of TPC, TFC, and TAC yields

increased with the increase in temperature and pressure. Although the SCE proved to be a promising alternative to extract bioactive compounds from plant materials, the supercritical technology is considered unfavorable due to high operational costs and the process's requirement to be operated at high pressures with exact process control. The accurate knowledge of solids' equilibrium solubility data in supercritical CO_2 in a wide range of temperature and pressure is crucial for designing supercritical extraction processes [36-38]. In most cases, these solubility data are not easy to predict because of the lack of reliable models for predicting solid equilibrium solubility in supercritical fluids and limited information on the physical parameter necessary for those available models [39, 40]. Therefore, modeling and correlation are required to develop a mathematical model that can describe the system based on a limited number of existing experimental data.

Many mathematical models have been developed to predict the phase behavior of solid-supercritical fluid systems [41, 42]. These models can also provide valuable context for understanding the dissolution phenomenon and can be used to predict solute solubility in supercritical CO_2 at desired pressures and temperatures. In this case, some of the developed models are purely empirical, although some may have a fundamental thermodynamic basis. A cluster analysis, density-based method, is an empirical or semi-empirical approach that relies on developing a relationship between a solid solute's solubility and the density of supercritical fluid based on the simple error minimization using the least-squares method. The correlation of solids' solubility in supercritical CO_2 based on density-based models provides a more straightforward way than the equations of state (EoS)-based models because it does not require critical and thermophysical properties of the involved solid [43].

Chrastil (1982) derived a density-based model by correlating solids and liquids' solubility in supercritical gases based on the supposition that one molecule of solute A associated with *k* molecules of solvent B to form a solvate complex AB_k in equilibrium with the system [35, 44-46]. This model has been widely applied to model solid solubility of phenolic compounds derived from natural matrices in supercritical CO_2 with satisfactory correlation results [47, 48]. Chrastil's model has linear (Eq. 7) and non-linear (Eq. 8) forms as follows:

$$\ln Y = k \ln \rho + \alpha/T + \beta \quad (7)$$

$$Y = \rho^k \exp(\alpha / T + \beta) \quad (8)$$

In the above expressions, Y is the solid solubility in a supercritical fluid or the number of bioactive compounds extracted from SNL by SCE (g/L), ρ is the density of CO_2 (g/L), T is the extraction temperature (K), k is the association number, α is a constant account to the heat of vaporization and solvation of the solute related to the set temperature. For simplification purposes, the mixtures of phenolics, flavonoids, and alkaloids present in the supercritical extracts were quantified as gallic acid, quercetin, and hyoscyamine equivalent, respectively. The density of CO_2 at various temperatures and pressures was determined by the Peng-Robinson EoS, while the adjustable parameters k, α, and β of the Chrastil' model were obtained by non-linear least-squares fitting of experimental data.

Figure 4 shows 3D scatter plots of experimental data (the extraction yield) in terms of TPC, TFC, and TAC as a function of CO_2 density and temperature. The corresponding Chrastil's model fits. The experimental data are denoted by solid circles, while the 3D wireframe plots represent non-linear least-squares model fits experimental data. It is shown

that extracts started to emerge as a specific CO_2 density was reached 200 g/L, and at higher temperatures, the extract can be obtained more. A similar phenomenon is also observed in other SCE related researches [45, 49, 50]. These results exhibited that the analyte's constant CO2 density, temperature, and vapor pressure have a parallel effect. The increase in temperature causes an increase in the analyte's vapor pressure, thereby increasing the solubility of the analyte in supercritical CO_2, and the extraction proceeds more effectively [34]. The exponential curves were observed from all extracts suggest that Chrastil's model can be used to describe the SCE of SNL.

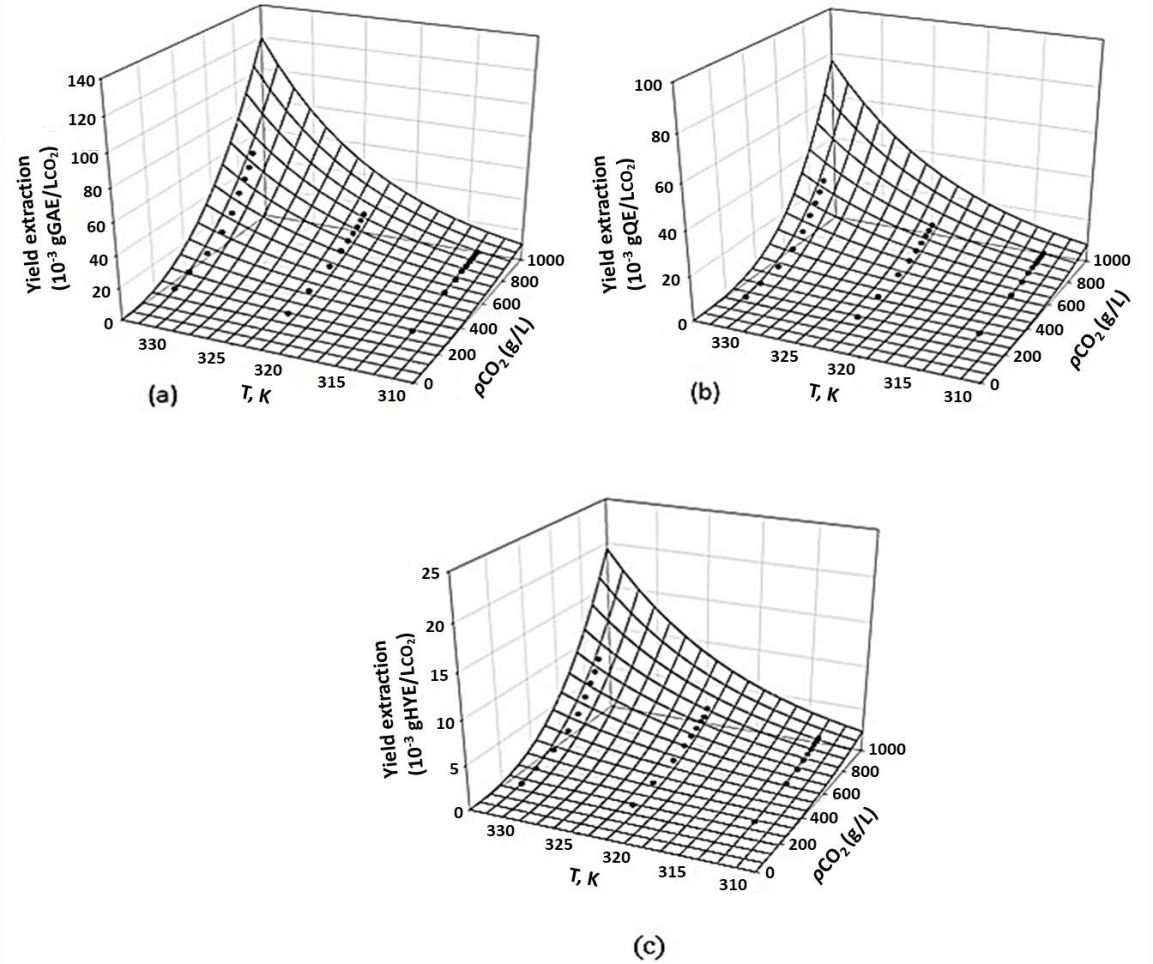

Figure 4. Extraction yields of bioactive compounds from SNL berries as a function of CO2 density and temperature in terms of (a) TPC, (b) TFC, and (c) TAC, and the corresponding Chrastil's model fits. Symbols represent experimental data. The yields of phenolics, flavonoids, and alkaloids were expressed as mg GAE/L CO2, mg QE/L CO2, and mg HE/L CO2, respectively.

The parameter *k* in Chrastil's model represents the number of supercritical CO_2 molecules involved in the solvated complex according to the following equilibrium reaction:

$$A + k\,CO_2 \leftrightarrow A(CO_2)_k \quad (9)$$

In most cases, the constant *k* is not an integer because the formation of solvated complexes $A(CO_2)_k$ is not always stable where some of the solvated compounds are less or more stable than others. As recorded in Table 3, the extracts (phenolic, flavonoid, and alkaloid) show a low *k* value. It indicates that only a slight amount of CO_2 molecules is needed to form the solvated complexes; hence the extraction precedes effectively. Of the three extracts, phenolic (TPC) has the lower *k* value (2.137), which indicates that phenolic is extracted the

most; this fact is consistent with the experimental result where extraction yield for phenolic (55.1677 mg/g) was the highest among the others. Alkaloid (TAC, k = 2.495) has a lower k value than that of flavonoid (TFC, k = 2.942); however, the extraction yield of the alkaloid (5.9460 mg/g) is lower than flavonoid (28.0308 mg/g). This deviation might arise due to alkaloid instability considering it has low thermal stability and tends to react with CO_2 to form carbonate salt [27, 51].

Table 3. Fitted parameters of Chrastil's equation.

Parameters	k	α	β	R^2	%AARD
Phenolic – TPC	2.137	-10,080	20.13	0.9878	14.17
Flavonoid – TFC	2.942	-8,840	12.01	0.9871	17.95
Alkaloid – TAC	2.495	-9,378	12.11	0.9822	26.18

Parameter α obtained by non-linear least-squares fitting of experimental data are presented in Table 3 with TPC, TFC, and TAC values are 10080, 8840, and 9378, respectively. TPC has the highest absolute α values; means that the temperature highly influences the TPC yield. Furthermore, a temperature increase of twenty degrees gives rise in TPC, TFC, and TAC yields approximately 6.0, 4.3, and 2.6-fold, respectively. Again, there is a deviation for TAC, which is due to its instability in CO_2. The negative value of α indicates an exothermic behavior of the supercritical extraction process.

The parameter β is associated with the number and molecular weights of the solute and supercritical fluid based on the following equation:

$$\beta = \ln(M_A + kM_{CO2}) + q - \ln(kM_{CO2}) \quad (10)$$

M_A and M_{CO2} are the molecular weights of bioactive compounds and CO_2, respectively, and q is a constant. Since the phenolics, flavonoids, and alkaloids were described as gallic acid, quercetin, and hyoscyamine equivalent; thus, the respective molecular weights are 170.12, 302.24, and 289.38 g mol^{-1}. Accordingly, the constant q for the phenolic, flavonoid, and alkaloid can be calculated to be 19.10, 10.81, and 10.82.

The objective function of the average absolute relative deviation (%AARD) between experimental and calculated values of the extraction yields was calculated by the following expression:

$$\text{AARD}\,(\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{y_{\text{exp},i} - y_{\text{calc},i}}{y_{\text{exp},i}}\right| \quad (11)$$

where $y_{exp,i}$ and $y_{calc,i}$ are the experimental and calculated extraction yields for experimental point i, in the unit of g/L CO_2, respectively, and n is the total number of experimental points. All extraction yields have an R^2 value close to 1, which indicates the apparent correlation between Chrastil's model to the resulting data. The R^2 value could not depict the goodness of correlation for each extract individually. The goodness of data correlation can be covered by using the AARD value. Between the three extracts, an alkaloid (TAC) has the highest AARD; this indicates the big deviation of the experimental and calculated data. The high AARD for TAC is obviously due to its instability in CO_2.

4. Conclusions

Supercritical carbon dioxide extraction on Solanum nigrum has been successfully conducted. The bioactive compounds present in the extract were identified and quantified. The

experimental designs have been performed using the Box-Behnken towards three parameters of temperature, time, and pressure. An empirical model, namely Chrastil's model, has been applied to correlate supercritical carbon dioxide's solubility as an extraction solvent with the extracted active compound. The Chrastil's adjustable parameters k, α, and β are consistent with their physical meaning and comparable to other systems.

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Conflicts of Interest

The authors declare no conflict of interest.

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