Extraction and solubility modelling of Sarawak Black Pepper Oil in Supercritical Carbon Dioxide

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Abstract. The solubility of Black Pepper Oil (BPO) was measured in Supercritical Carbon Dioxide (SC-CO_2). The temperatures and pressures of the extraction were chosen in the range of 313 – 333K and 100 - 300 bars, respectively. The solubilities attained ranged between 0.27×10^{-5} to 2.88×10^{-5} g extract/g CO_2. 5 different empirical models were selected to predict the solubility of BPO in SC-CO_2. Among the 5 empirical models, Belghait model resulted in the lowest but best absolute average relative deviation (AARD) of 14.90%.

1. Introduction

Sarawak Black Pepper has been acknowledged as a premium black pepper in the global market due to its high quality and is therefore exported worldwide either in the form of black pepper powder, or as black pepper berries. Although pepper is said to be one of Malaysia's staple crop, more than 95% of it is grown in Sarawak. A significant growth in Malaysia's pepper production, from 24,227 tonnes in 2010 to 30,804 tonnes in 2020 was seen [1]. Therefore, it is no surprise that the Sarawak State Government has great interest in increasing the economic value of black pepper by developing value-added products derived from black pepper's essential oil and oleoresin, which are two of the many black pepper bioactive compounds which have therapeutic and medicinal benefits [2].

The numerous benefits associated with supercritical fluid technology (SFT) has led to a growing interest amongst researchers in adopting the technology for the extraction of bioactive compounds from natural matrices. As oppose to conventional solvent extraction methods, the moderate operating conditions applied helps prevent thermal decomposition especially when handling thermolabile bioactive compounds [3]. In addition, the SFE process has higher selectivity, higher solubility, shorter extraction times, as well as has been recognized as a green extraction process when carbon dioxide is taken as a solvent. These advantages allow it be highly regarded specifically in the pharmaceuticals, nutraceuticals, food, and cosmetics industry; just to name a few [4]. Similar to any other extraction process, parameters that have been proven to influence the results of the SFE include operating conditions, particle size, choice of solvent, presence of co-solvent, and polarity of the compound of interest. Nevertheless, it is important to note that as SFE is a mass-transfer related process, the yield is limited by the solubility of the targeted compound. As such, in order to further improve the yield of the SFE process and improve its efficiency, solubility data of the solute of interest should best be known.

Experimental efforts to collect solubility data at a wide range of operating conditions are often labor-intensive, costly, and time-consuming. Thus, to facilitate the data collection process, predictive solubility modeling, which includes empirical, and thermodynamic modeling [5], are two of the most common options available with the former being favored more by researchers due to the its simplicity as oppose to the latter. The simplicity of empirical models allows for relatively quick computation, involving only pressure and temperature parameters presented in various mathematical equations. To date, there exists about 23 empirical models that have been developed by various researchers to aid in predicting solubility [6]. To the best of our knowledge, experimental studies that have been carried out concerning the extraction of black pepper by supercritical CO_2 (SC-CO_2) is rather scare [7, 8] while it is also safe to report that no solubility data of black pepper oil is has been presented in literature thus far. In line with this understanding, the present study aims to study the effects of various combinations of pressure and temperature in extracting Sarawak Black Pepper Oil, subsequently modeling its solubility using 5 different empirical models.

2. Materials and Methodology

2.1. Methodology

2.1.1. Supercritical Fluid Extraction of Black Pepper Oil (BPO)

Experimental works to extract essential oil from Sarawak Black Pepper in SC-CO2 was conducted using a set of apparatus, made up of the Spe-ed SFE Extractor (manufactured by Applied Separations, Inc. USA), a Carbon Dioxide Cylinder Tank to supply CO_2 to the extractor, a chiller for CO_2 liquefaction to take place, and lastly, a high pressure syringe pump (manufactured by Applied Separations, Inc. USA) Before pressurizing the vessel, the temperature of the chiller has to drop to 0ºC as the circulating coolant has to be maintained at 0ºC. The chiller bath contains a mixture of ethylene glycol which is an anti-freeze reagent, and water at a ratio of 1:1. A dynamic experimental method was undertaken where 3 different temperatures of 313, 323, 333K and 5 different pressures of 100, 150, 200, 250, and 300 bars, which sums up to 15 sets of operating condition was chosen. Extraction at each of the 15 operating conditions combination was performed twice to ensure repeatability of the process. At each set of operating condition, approximately 630.0g grounded black pepper (supplied by MicroKlean - Quality Spices, with approximately 1mm diameter) was carefully weighed and loaded into the 25 kg extraction vessel. Subsequently, the temperature of both vessel and oven were allowed to equilibrate towards the set operating value, after which pressurization of the vessel towards the desired operating pressure begins. Static time of 15 minutes was necessary to allow saturation of SC-CO2 within the vessel. Subsequently, the heated micro-metering valve is opened to allow the black pepper extracts to flow continuously into a clear collection vial until the accumulated yield reaches a plateau. As the SC-CO2 flows through the black pepper sample from bottom to top, the vessel becomes saturated with the extracted black pepper oil. The black pepper extracts were weighed using an analytical balance after every 30 minutes until constant readings are achieved, indicating that maximum equilibrium has been achieved. The flowrate of SC-CO2 was set at approximately 2.5 L/min by observing the readings on the flowmeter and is closely monitored by controlling the opening of the restrictor valve. Figure 1 shows the Process Flow Diagram of the SFE process that was carried out. The amount of CO_2 consumed was computed using Microsoft Excel, based on the flowrate and the density at the respective operating conditions. A graph of accumulated black pepper weight versus mass of CO_2 consumed was plotted for each operating condition investigated. The computed yield of the extraction is presented in Table 1.

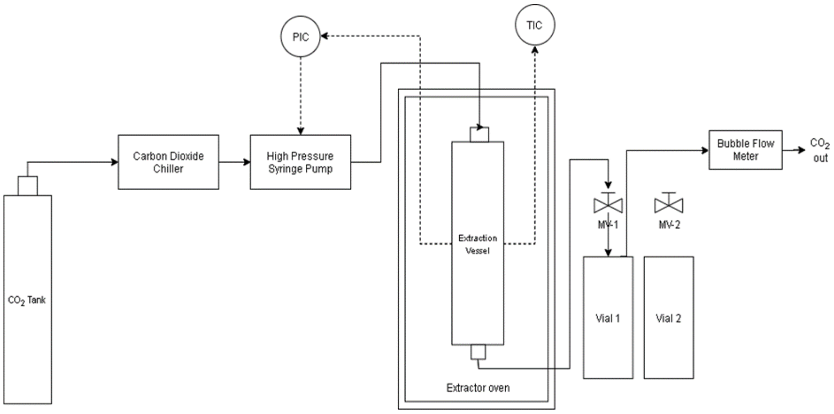

Figure 1: Process Flow Diagram (PFD) of Spe-ed SFE Apparatus to measure solubility of Black Pepper extract; MV – metering valve, PIC – Pressure indicator, TIC – Temperature indicator

The slope of each accumulated weight versus CO_2 consumed represents the solubility of BPO. The method of obtained solubility through the graph slope has been adopted by various researchers and has been proven reliable. Thus, solubility is defined by Equation 1 below.

$$Solubility, y_2 = \frac{Accumulated\ mass\ of\ oil\ (g)}{Total\ mass\ of\ CO_2\ consumed\ (g)} \tag{1}$$

2.1.2. Empirical Modeling

As discussed in Section 1, predictive solubility models are handy tools that can replace the laborious, costly, and time-consuming manual method of collecting solubility data. This work compares the performance of 5 different types of empirical models out of the 22 models that are present to date. These 5 empirical models are presented in Table 1, accompanied by its respective equations. The performance of the empirical models were gauged according to its Absolute average relative deviation (AARD) as shown by Equation 2, whereby a lower AARD indicates better performance.

$$AARD\ (\%) = \frac{100}{N}\sum_{i=1}^{N}\frac{y_{i,exp} - y_{i,calc}}{y_{i,exp}} \tag{2}$$

Table 1: Empirical Models and Accompanying Equations.

Empirical Model	**System**	**Model Equation**	**Eq.**	**No. of Parameters**	**Ref.**
Belghait	binary	$\ln(y_2) = a + b\rho + c\rho^2 + d\rho T + eT + fT^2 + g\ln\rho + \frac{h}{T}$	(3)	8	[6]
Gordillo	binary	$\ln(y_2) = a + bP + cP^2 + dPT + eT + fT^2$	(4)	6	[9]
Moussa	binary	$\ln(y_2) = a + b\rho + c\rho^2 + d\rho T + e\frac{T}{\rho} + f\ln\rho$	(5)	6	[10]
Sodeifian	binary	$\ln(y_2) = a + b\frac{T}{\rho} + c\ln(\rho T) + d(\rho\ln\rho) + eP\ln T) + f\frac{\ln(\rho)}{T}$	(6)	6	[11]
Khansari	binary	$\ln(y_2) = \frac{a}{T} + bP + c\frac{P^2}{T} + d\ln\rho_1 + eP\ln\rho_1$	(7)	5	[12]

2.1.3. Characterization of Black Pepper Extract

The collected black pepper extract comprised of black pepper oil which was seen at the early stages of the extraction, and oleoresin, which was released only later in the experiment. Once extraction was completed and the collection vial was left idle for a few days, a layer of oleoresin settled at the base of the collection vial and a layer of black pepper oil was seen at the top. Characterization of the two layers were carried out via two different methods. GC-MS analysis was undertaken to test the contents of black pepper oil whereas HPLC analysis was applied to analyse the Oleoresin layer. The composition of selected components found through GC-MS analysis of the Black Pepper Oil was determined through the area under the curve of the chromatogram. Component identification was processed by the built-in GC-MS library.

HPLC analysis, however, involved the preparation of a standard piperine solution of 1 mg/mL that was made by simply dissolving 10 mg of piperine into 10 ml of HPLC-grade methanol, thus obtaining a calibration curve. The 1 mg/mL solution was further diluted with the same solvent to form several working standard solutions of different concentrations; 0.005%, 0.01%, 0.02%, and 0.1% concentrations [13] Prior to sending the oleoresin (OR) sample for analysis, it was necessary to soften the oleoresin by placing it in a warm 40^0C water bath, which was later stirred to attain a homogenized solution. 0.2g of the softened oleoresin was accurately weighed and subsequently transferred into a 100 mL volumetric flask where HPLC-grade ethanol was added into the volumetric flask. The mixture was shaken well to form a well-mixed working solution

3. Results and Discussion

3.1. Solubility of Black Pepper Oil

The solubility of Black Pepper Oil was obtained at 15 combinations of operating conditions and the results were presented in Table 2, ranging from 0.27 x 10^{-5} to 2.88 x 10^{-5} g extract/g CO_2. The highest solubility of 2.88 x 10^5 g extract/g CO_2 was obtained at the highest operating condition (300 bars, 60^0C); similar to a study carried out by Kumoro et al. on the solubility of piperine [8], and also solubility studies that were carried out by several other researchers [4, 14] concerning the solubility of bioactive compounds in general. Generally, an increasing trend in solubility was observed at isothermal conditions except for two anomalies that were detected at 200 bars, 60^0C and 300 bars, 50^oC as seen in the graph plotted in Figure 2. This increasing trend in solubility is in line with the theoretical understanding that solubility increase with pressure due to an increased density, subsequently leading to a greater solvation power of SC-CO_2. In other words, as the operating pressure increases, there is more intermolecular interaction between the supercritical solvent solute molecules. The anomalies in the results may be due to experimental-related errors such as needle blockage, whereby it caused the chilling of collection vial over periods longer than 30 minutes. Consequently, there was an interrupted flow of CO_2 from the delivery tube towards the flowmeter, which resulted in an accumulation of CO_2 within the collection vial. Besides, a second plausible explanation for the anomaly reported was the timeline of the extraction. At higher operating conditions, specifically 300 bars, the extraction was spread out over two days. In such circumstances, the first yield measurement obtained on the second day was greater than that of the last measurement obtained before the experiment was halted on the previous evening. This could be an indication that a small amount of oil collected from the previous day was not fully released into the collection vial albeit the fact that the valve was fully opened during the depressurization process during equipment shutdown procedures.

Table 2: Solubility of BPO at various operating conditions.

Pressure (bar)	Temperature (^{0}C)	Density (kg/m^3)	Solubility (10^{-5} g oil/g CO_2)
100	40	628.7	0.2700
100	50	384.4	0.3300
100	60	290.0	1.1500
150	40	780.3	1.8900
150	50	699.8	1.9100
150	60	603.9	2.5400
200	40	839.9	2.2600
200	50	784.4	2.4600
200	60	723.8	2.3900
250	40	879.6	2.0300
250	50	834.4	2.0900
250	60	786.8	2.4100
300	40	910.0	2.7800
300	50	870.6	2.7000
300	60	863.2	2.8800

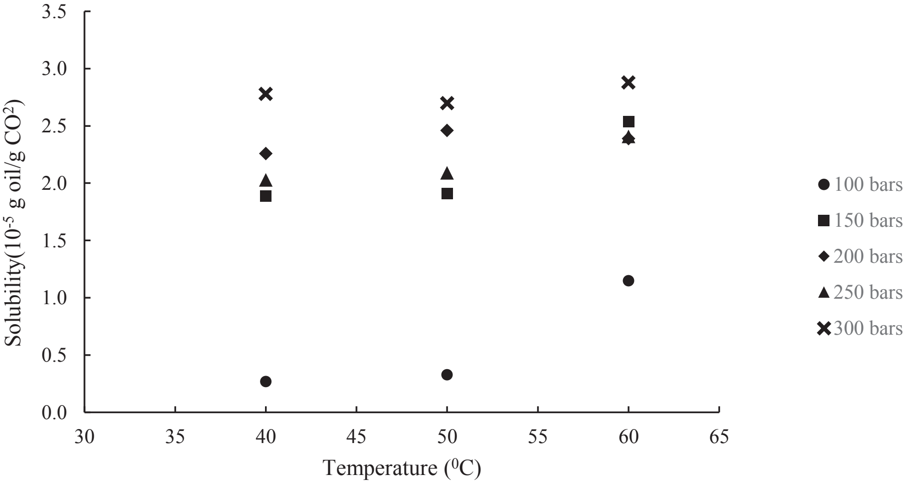

Figure 2: Solubility of BPO at isobaric conditions

3.2. Correlative performance of 5 Empirical Models

The 5 empirical models presented in Section 2.1.2 were adopted in this study to model the experimental solubility presented in Table 2. The regression process to obtain the AARD (%) was carried out in Microsoft Excel and the results attained showed that the 8-parameter Belghait model performed best among the 5 models. The said model was proposed in year 2018 by Belghait et. al [6] and stood out among 21 other empirical models in regressing a comprehensive data set which comprised of 210 different pharmaceutical compounds and 5500 data points, respectively. In a recent study by Sajadian et. al [15] in correlating the solubility data set of *Lenalidomide* involving 30 different empirical parameters, the Belghait model, resulted in the lowest AARD of 7.516% while the Sodeifian model resulted in a higher AARD of 11.45%. A separate study by Sodeifian et al. in correlating the solubility of rivaroxaban [11], inferred that a model with greater number of parameters tend to be superior to models that has a smaller number of parameters. Based on Table 3, this study, too, proves that the correlative performance of the models decreased with the number of parameters involved in the associated model equation. This was also evident in other studies [6] concerning predictive solubility modeling of bioactive or pharmaceutical compounds in SC-CO_2.

Table 3: Computed AARD (%) for BPO solubility using 4 empirical models.

Empirical Model	no. of parameters	AARD (%)	Reference
Khansari	5	33.39	[12]
Gordillo	6	26.22	[16]
Moussa	6	22.89	[10]
Sodeifian	6	15.76	[11]
Belghait	8	14.90	[6]

3.3. Qualitative Analysis of Extract by HPLC analysis

After the extract was allowed to rest in the collection vial, two distinct layers were formed; the oil, and the semi-solid oleoresin layer. The oleoresin layer was qualitatively analysed by means of High-Performance Liquid Chromatography (HPLC), whereby the presence of Piperine, being the targeted compound was confirmed as shown in Figure 4. It was found that 3 common compounds namely, Caryophyllene, Caryophyllene Oxide, and Humulene, were present in all of the extracted oil layers that underwent Gas Chromatography-Mass Spectrometric (GC-MS) analysis. Another similarity between all chromatograms obtained was that the highest peak belonged to Caryophyllene. It was not surprising that caryophyllene was present in all the samples tested as it is the compound that contributes to the spiciness of Black Pepper. This finding is in good agreement with other publications which also reported that the area under the curve for Caryophyllene was highest among all the other components [7, 17, 18].

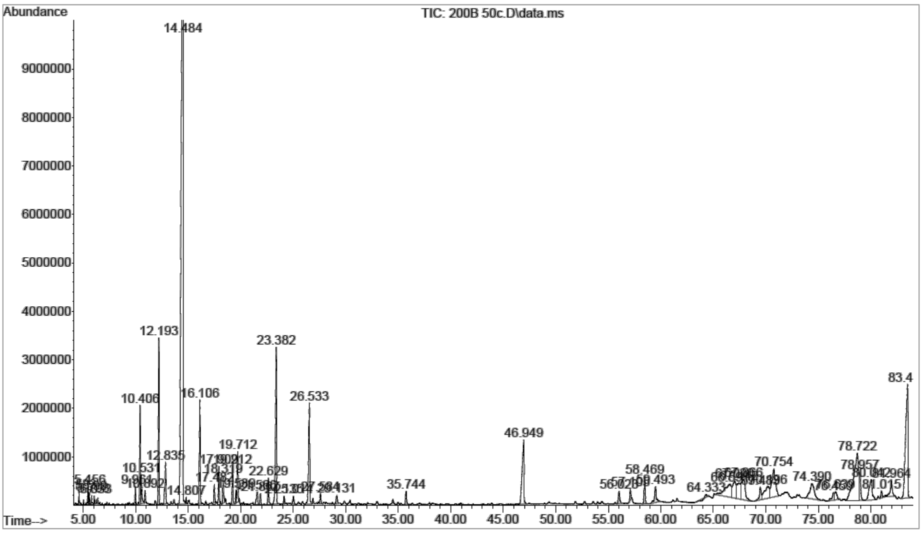

Figure 3: Sample of GC-MS chromatogram of Black Pepper Oil layer at 200 bars, 50^0C.

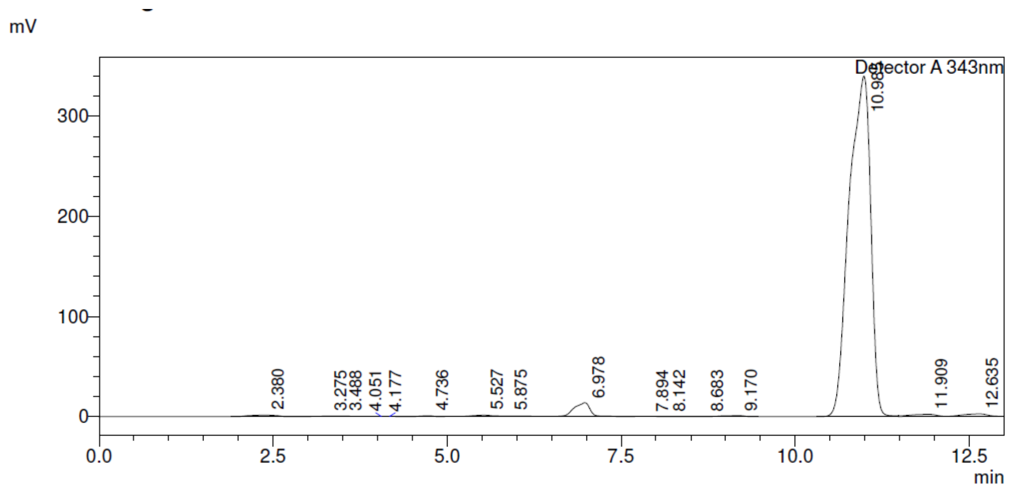

Figure 4: Sample of HPLC chromatogram of Black Pepper Oleoresin Layer at 200 bars, 50^0C.

4. Conclusion

The solubility of Black Pepper Oil in Supercritical Carbon Dioxide was determined at temperatures ranging between 313 – 333K and 5 different operating pressure between 100 - 300 bars. The results attained were generally in line with past research work where solubility increases with temperature at isobaric conditions. Moreover, the aforementioned results were successfully correlated using 5 different empirical models namely Khansari, Gordillo, Moussa, Sodeifian, and Belghait model. The 8-parameter Belghait model performed best with a lowest computed AARD (%) of 14.90% while the 5-parameter Khansari model resulted in the highest AARD (%) of 33.39%.

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