Supercritical carbon dioxide extraction of astaxanthin from Paracoccus NBRC 101723: Mathematical modelling study

Jyoti A. Chougle, Sandip B. Bankar, Prakash V. Chavan, Vandana B. Patravale & Rekha S. Singhal

To cite this article: Jyoti A. Chougle, Sandip B. Bankar, Prakash V. Chavan, Vandana B. Patravale & Rekha S. Singhal (2016): Supercritical carbon dioxide extraction of astaxanthin from Paracoccus NBRC 101723: Mathematical modelling study, Separation Science and Technology, DOI: 10.1080/01496395.2016.1178288

To link to this article: http://dx.doi.org/10.1080/01496395.2016.1178288
Supercritical carbon dioxide extraction of astaxanthin from Paracoccus NBRC 101723: Mathematical modelling study

Jyoti A. Chougle, Sandip B. Bankar, Prakash V. Chavan, Vandana B. Patravale, and Rekha S. Singhal

ABSTRACT

Astaxanthin (AX) is a secondary metabolite that accumulates inside the cell during Paracoccus fermentation. The fermentation biomass was extracted using supercritical carbon dioxide (SC-CO₂). The solubility parameter of AX, CO₂ and entrainer solvents was calculated and validated with experimental results. The pressure and particle size of the biomass had a significant effect on the extraction of AX. A maximum recovery of 963.33 μg/g of AX was obtained after SC-CO₂ extraction at 40°C, 350 bar and a run time of 60 min with ethanol (20% v/w) as an entrainer. Further, the experimental data has been modelled using non-linear regression analysis method.

Introduction

Carotenoids are naturally occurring pigments having yellow, orange or red colour. They are one of the most complex classes of natural colours with more than 750 molecules isolated and structurally characterized till date. Carotenoids exhibit antioxidant properties, which enables them to prevent the accumulation of harmful reactive oxygen species (ROS) generated during cellular metabolism as well as chaotrope-induced and other stresses. They are naturally synthesized as hydrocarbons (carotenes) and their oxygenated derivatives as xanthophylls. Xanthophylls contain various oxygenated functional groups, making them structurally and functionally diverse carotenoid pigments.

Astaxanthin (3,3′-Dihydroxy-β,β-carotene-4,4′-dione) is a subclass of xanthophylls, commonly known as ketocarotenoid. Astaxanthin (AX) imparts higher antioxidant activity and is comparatively more polar than other carotenoids. The complex nature of AX molecule poses difficulty in its chemical synthesis, which yields a mixture of configurationally different isomers. AX production has been exclusively restricted to the few microorganisms where it is present as a secondary carotenoid. Microbial pigments play multifarious roles in the natural ecophysiology of the producing species and, indeed, in terms of biotechnological application. A number of algae, yeast and a few bacterial sources producing AX have been isolated and studied. At present, the emphasis is mainly on algal and yeast sources. However, their commercial production requires a higher incubation time along with cumbersome and expensive downstream processing. Hence, bacterial sources such as Paracoccus species offer an advantage due to lower fermentation time. Besides, Paracoccus MBIC 01143 increased the accumulation of AX to almost two-fold when compared with other strains.

AX is an intracellular pigment where it is naturally present and serves its various functions. Hence, the disruption of the cell wall is an essential step towards the extraction of AX. The tough sporopollenin cell wall in the cysts of Haematococcus pluvialis and the thick cell wall of Phaffia hinder AX extraction. A number of physical processes such as sonication, homogenization, chemical processes involving solvents and acids, and biological processes like enzymatic cell wall disruption using lytic enzymes have been used for extraction of AX. Sonication can be a very efficient technique since it extracts both low molecular weight solutes and high molecular weight compounds such as AX using chaotropic substances, solvents or supercritical extraction. The nature and permeability of cell wall majorly influence the method of extraction. Recently, the focus has shifted towards green technologies of extraction due to which supercritical fluid extraction (SCFE) is gaining popularity.
Conventionally, the extraction of intracellular AX is carried out using solvent extraction methods. Most of these solvents are not suitable for food and pharmaceutical applications. Hence, the use of GRAS (generally recognized as safe) solvents such as ethanol or the use of solvent-free technologies such as SCFE is considered to be superior over conventional techniques. SCFE uses carbon dioxide as a solvent in its supercritical state (SC-CO$_2$) above 31.1°C and 74 bar, and is generally suitable for the extraction of thermally sensitive molecules that are non-polar. The lower viscosity and higher diffusivity of supercritical fluid facilitate mass transfer, thereby decreasing the overall extraction time. The supercritical medium can be easily separated from the extraction products, thus eliminating any additional steps of separation. Further, it does not generate any chemical waste, making it a green technology. Carbon dioxide (CO$_2$) is not only readily available at high purity, but also safe to handle. These features make it an apt solvent for supercritical extraction process.

The use of SCFE for the extraction of AX from *Paracoccus* is limited and its literature is scarce. Hence, the present work was undertaken to describe the supercritical extraction of AX from *Paracoccus* MBIC 01143. The operating parameters like pressure, temperature, particle size and time of extraction have been optimized for higher extraction of AX. The effect of co-solvents on the extraction efficiency of AX was also studied using the Fedor solubility parameter approach. The one-factor at-a-time studies have been used effectively to optimize the production of biotechnologically important substances. However, due to the interaction of process variables that typify biological complexity, modelling approaches can represent a more direct and effective means to understand the system. Hence, an attempt has been made to model the experimental data using non-linear regression analysis within an ambit of operating parameters.

**Materials and methods**

**Materials**

All the chemicals and solvents used in the present study were of AR grade and purchased from S.D. Fine-Chem. Ltd, Mumbai, India. Methanol used for AX analysis was of HPLC grade (Merck, Canada). CO$_2$ cylinders were supplied by Bombay Carbon Dioxide, Mumbai, India. Standard AX (98% w/w) was obtained as a gift sample from DSM Nutritional Products Ltd., Switzerland.

**Bacterial strain and fermentation medium**

*Paracoccus sp.* NBRC 101723 (formerly available as MBIC 01143, also classified as *Agrobacterium aurantiacum*) was procured from Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Chiba, Japan. The fermentation medium composition used for maintenance and production contained glycerol (2 g/L), soy peptone (39.3 g/L) and NaCl (22.3 g/L) at pH 7.5 ± 0.2. The batch fermentation was carried out at 20 ± 2°C for 96 h on a rotary shaker at 180 rpm. The experiments were carried out in 250 ml Erlenmeyer flasks with a 100 mL working volume.

**Conventional solvent extraction**

The conventional extraction of AX was performed as per the previous study. The harvested cells from the fermentation broth were dried and then suspended in acetone and vortexed. The mixture was centrifuged at 10000 g for 5 min. The supernatant containing extracted AX was collected and the pellets were re-extracted with acetone. The supernatants were collected from pellets after repeated cycles of extraction till the biomass was colourless. The samples were pooled for analysis of AX content by HPLC.

**SC-CO$_2$ extraction of AX**

The SCFE was carried out using laboratory-scale supercritical equipment (Thar SFE-500) of Thar Technologies, Inc. PA, USA. High-pressure extraction vessel (SS 316 insulated) was packed with a cotton plug at the bottom, followed by a layer of SPEED matrix (a hydromatrix and dispersing agent) to eliminate the dead volume of the extraction vessel. A known amount of dried biomass layer was placed in the vessel and covered with a cotton plug. The vessel was packed firmly to ensure that CO$_2$ diffused uniformly through the sample matrix. The vessel was kept in place and a thermocouple was connected to the vessel body. The system was equipped with an automated back-pressure regulator, which maintained the pressure at the desired level. A pressure-tight cyclone collector (made of SS 316) was used to collect the extract from CO$_2$. The operating conditions of SC-CO$_2$ extraction were optimized as described next.

**Effect of pressure**

Optimization of the pressure for maximum extraction of AX was done by varying the pressure from 150 to 350 bar. The flow rate of SC-CO$_2$ temperature of extraction
and time of extraction were kept constant at 15 g/min, 35°C and 60 min, respectively. The SC-CO₂ extracts so obtained were estimated for AX by HPLC.

**Effect of temperature**

To evaluate the effect of temperature, AX extraction was carried out under the optimized pressure established from the prior set of experiments. The temperature was varied from 35°C to 65°C with a flow rate of 15 g/min for 60 min. The SC-CO₂ extracts were then estimated for its AX content.

**Effect of particle size of biomass**

The particle size of the dried biomass was optimized for higher AX extraction. The size of the dried biomass was varied by grinding it and passing through the sieves. The ground biomass was sieved to obtain coarse (3 mm) and finely ground powder (0.1 mm). A control experiment was performed without any grinding of the dried biomass.

**Effect of time**

The extraction time required for higher AX release was estimated by carrying out extraction for different time intervals (30–120 min). The flow rate of SC-CO₂ was kept constant at 15 g/min. Other experimental parameters like pressure, temperature and particle size were kept at their optimum. The extract obtained after SC-CO₂ extraction was further analysed by HPLC.

**Effect of entrainer**

Supercritical CO₂ is highly non-polar in nature. When a polar solvent (entrainer) is mixed with SC-CO₂, the polarity of the supercritical CO₂ is altered, which in turn increases the solubility of polar compounds. Hence the dried biomass of AX was dampened with entrainers, which are chaotropic alcohols such as methanol, ethanol and isopropanol at 10% and 20% v/w and extracted using SC-CO₂ under previously optimized conditions. The SC-CO₂ extracts thus obtained were then estimated for the content of AX by HPLC. The control experiments with conventional solvent (acetone) extraction were also performed to compare the extraction efficiency of SC-CO₂.\(^{[13]}\)

**Analysis of AX by HPLC**

The extracted AX was dissolved in acetone and analysed by HPLC (Jasco, Tokyo, Japan) using Waters Spherisorb C18 reverse-phase column (4.6 × 250 mm, 5 μm). The HPLC method reported by Asker et al.\(^{[22]}\) was used for the quantification of AX with slight modifications. The mobile phase used was a mixture of methanol and water (95: 5 v/v) with a flow rate of 1 mL/min. AX was detected by measuring the absorbance at 476 nm. A standard curve of absorbance vs. concentration of pure AX was plotted using a concentration range of 10–100 μg/mL (R² = 0.9986), which was used to estimate the AX in the SCFE extracts.

**Estimation of solubility parameters**

One of the ways to have a detailed insight into the solubility of a solute in supercritical fluid is the estimation of its solubility parameters. Solubility parameter is very helpful to predict the solubility of a solute in supercritical fluids.\(^{[23]}\) The estimation of solubility parameter provides a semi-quantitative evaluation of experimental conditions to be selected for optimized extraction conditions. The solubility parameter (δ) of a supercritical fluid can be estimated by using the following equation:\(^{[24,25]}\)

\[
\delta = \frac{1}{2} \left( \frac{\text{cal}}{\text{cm}^3} \right)^{1/2} = \frac{1}{2} \left( \frac{1}{\rho_{r, SF}} \right)^{1/2} = 0.47 \rho_{r, SF} \delta_c^{1/2}
\]

where \(P_c\) is the critical pressure (bar), \(\rho_{r, SF}\) is the reduced density of the supercritical fluid (g/cm³) and \(\rho_{r, L}\) is the reduced density of liquid state (g/cm³). This equation reflects the variation of the solvent power of the supercritical fluid as a function of density. Solubility parameter (δ) of a given solute can be estimated by using the Fedors group contribution method when the solute molecular structure is known.\(^{[26]}\) Table 1 shows the estimation of the solubility parameter of AX by using the Fedors method.\(^{[26]}\) The solubility parameters of AX were calculated using the following equation:

\[
\delta = \frac{1}{2} \left( \frac{\text{cal}}{\text{cm}^3} \right)^{1/2} = \left[ \frac{\sum (\Delta f)}{\sum (\Delta V)} \right]^{1/2}
\]

where

<table>
<thead>
<tr>
<th>Table 1. Solubility parameter of AX using Fedors group contribution.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>CH3</td>
</tr>
<tr>
<td>CH2</td>
</tr>
<tr>
<td>C=O</td>
</tr>
<tr>
<td>CH</td>
</tr>
<tr>
<td>CH2</td>
</tr>
<tr>
<td>CH=</td>
</tr>
<tr>
<td>CH2=</td>
</tr>
<tr>
<td>6-membered ring</td>
</tr>
<tr>
<td>Conjugated double bonds</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
\[ \delta \text{ (cal/cm}^3) \] is the solubility parameter of solute at temperature \( T_i \) (cal/cm\(^3\)^\(1/2\))

\( \delta_1 \) is the solubility parameter of solute at temperature \( T_1 \) (cal/cm\(^3\)^\(1/2\))

\( \delta_2 \) is the solubility parameter of solute at temperature \( T_2 \) (cal/cm\(^3\)^\(1/2\))

Hildebrand\(^{30}\) proposed the square root of the cohesive energy density as a numerical value, indicating the solvency behaviour of a specific solvent. The solubility parameter of the entrainer solvent, \( \delta \), at different temperatures and pressures was calculated using the following equation:

\[
\delta = \left( \frac{\sum (\Delta E_i)}{V} \right)^{1/2} = \left( \frac{\Delta H_{vap} - RT}{V} \right)^{1/2}
\]

where

\( \Delta E_{vap} \) = summation of all cohesive energy, (cal/mol)

\( \Delta H_{vap} \) = heat of vaporization of solvent, (cal/mol)

\( R \) = gas constant, 1.987 cal/mol K

\( V \) = molar volume, (cm\(^3\)/mol)

### Table 2. Solubility parameters of AX at different temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Solubility parameter ( \delta ) (Mpa(^1/2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>20.33</td>
</tr>
<tr>
<td>40</td>
<td>20.29</td>
</tr>
<tr>
<td>45</td>
<td>20.24</td>
</tr>
<tr>
<td>50</td>
<td>20.19</td>
</tr>
<tr>
<td>55</td>
<td>20.14</td>
</tr>
<tr>
<td>60</td>
<td>20.08</td>
</tr>
<tr>
<td>65</td>
<td>20.03</td>
</tr>
<tr>
<td>70</td>
<td>19.98</td>
</tr>
<tr>
<td>75</td>
<td>19.93</td>
</tr>
</tbody>
</table>

The compressibility factor for entrainer solvent at varying pressure and temperature conditions obtained from Reid and Sherwood\(^{31}\) was used to calculate \( \Delta H_{vap} \) under different conditions.

### Results and discussion

#### Estimation of solubility parameters

The solubility parameter was evaluated to select appropriate experimental conditions for optimization of the SC-CO\(_2\) protocol. The solubility parameter theory qualitatively explains the effects of temperature and density (or pressure) on solubility in supercritical fluids. According to this theory, a higher solubility is achieved when the solubility parameters of the solute and solvent are closer to each other.\(^{32}\) Hence the solubility parameters were calculated to understand the solubility of AX in the SC-CO\(_2\) with respect to pressure and temperature. Figure 1 shows the dependence of solubility parameters of AX and SC-CO\(_2\) as a function of temperature and pressure. It can be seen that the solubility parameter of AX remains fairly constant as a function of temperature. A similar trend is followed by the solubility parameter of SC-CO\(_2\) at higher pressure (300–400 bar). However, it is evident that the solubility parameter of SC-CO\(_2\) is a strong function of pressure. The value of solubility parameter is 12.4 Mpa\(^1/2\) and 16.35 Mpa\(^1/2\) at 35°C for 100 and 300 bar, respectively. An increase in the pressure shows the value of solubility parameter of SC-CO\(_2\) close to the solubility parameter of AX, which results in the enhancement of extraction efficiency. Thus, according to the solubility parameter approach, the favourable condition for extraction of AX
would be at 350 bar and 35°C. The practical feasibility of AX extraction with initial conditions (temperature 35°C, pressure 350 bar and run time of 60 min) was validated with the experimental data.

**Supercritical fluid extraction of AX**

The selection of the operating conditions depends on the compound to be extracted, in particular its molecular weight and polarity, which influence the extraction process. Factors such as pressure and temperature need to be optimized during SC-CO$_2$ extraction.

**Effect of pressure on SC-CO$_2$ extraction**

The extraction pressure is the most significant process parameter of SC-CO$_2$ extraction and is used to adjust the selectivity of the supercritical fluids (SCF). In general, an increase in the pressure enhances the solvent power and decreases extraction selectivity.$^{[17]}$ Hence, the effect of extraction pressure was evaluated at a constant temperature of 35°C for 60 min, using SC-CO$_2$ at a flow rate of 15 g/min. Experimental runs at different pressures were conducted to study the effect and select the optimum extraction pressure (Fig. 2a). The extraction efficiency was found to increase with an increase in pressure up to 300 bar. The maximum AX recovery at 300 bar was found to be 730 μg/g. The AX extraction remained unaltered beyond 300 bar. These results are in accordance with Sajilata et al.$^{[27]}$ who reported an increase in zeaxanthin recovery up to 300 bar and beyond which it decreased with an increase in the pressure of the system. At higher pressure, CO$_2$ becomes highly compressed and solvophilic interactions are overcome by solvophobic interactions between the solute and the solvent, which result in repulsing solute–solvent interactions. This incidentally induces complex extraction and minimizes the extraction efficiency of the solute.$^{[33]}$

**Effect of temperature on SC-CO$_2$ extraction of AX**

The selected temperature range for extraction should be in the vicinity of the critical point and as low as possible to avoid degradation in case of thermolabile molecules. An increase in temperature reduces the density of SC-CO$_2$ (for a fixed pressure), thus reducing the solvent power of the supercritical solvent; however, it increases the vapour pressure of the compounds to be extracted. Therefore, the tendency of these compounds to get extracted in the fluid phase is increased.$^{[17]}$ The effect of temperature (35–55°C) at 300 bar on AX recovery was investigated using SC-CO$_2$ (Fig. 2b). A

![Figure 2. Effect of pressures (a), temperature (b), particle size (c) and extraction time (d) on supercritical fluid extraction of AX.](image-url)
very small increase in the recovery of AX was achieved at 40°C (769 μg/g). The AX extraction remained unaltered after 40°C. The optimum conditions for the extraction vary from one source to another and therefore have to be optimized separately. Nobre et al.\textsuperscript{[14]} reported the use of SC-CO\textsubscript{2} extraction for the recovery of AX from \textit{Haematococcus pluvialis} to result in 92% w/w carotenoid recovery at 300 bar/60°C. On the contrary, Lim et al.\textsuperscript{[15]} achieved maximum yield of both carotenoids and AX from \textit{Phaffia rhodozyma} to be 84 and 90% w/w, respectively, at 40°C and 500 bar.

**Effect of particle size and time of extraction**

Apart from pressure and temperature, the particle size of matrix and time of extraction are important process parameters to be considered for SCFE process optimization.\textsuperscript{[17]} Since carotenoids are intracellular, pretreatments such as crushing, freeze drying and enzymatic cell degradation aid in the extraction process. In the present study, two particle sizes were used for extraction, viz. coarsely ground (0.3mm) and finely ground (0.1 mm), which were compared with the intact biomass. The biomass was finely ground just before loading it into the extraction vessel to avoid degradation. The extraction increased from 749 μg/g (using 0.3 mm) to 844 μg/g (0.1 mm) for the finely ground particles (Fig. 2c).

The effect of extraction time was also evaluated at a constant temperature of 40 °C and pressure of 300 bar using SC-CO\textsubscript{2} at a constant flow rate of 15 g/min. A maximum recovery of 844.67 μg/g of AX was obtained after 60 min of extraction (Fig. 2d), beyond which no significant increase in recovery was observed. Thus, an extraction time of 60 min was found to be suitable to extract AX under the tested conditions.

**Effect of co-solvents**

The non-polar nature of pure SC-CO\textsubscript{2} is unsuitable for the extraction of slightly polar solutes due to low solute solubility. The polarity of pure SC-CO\textsubscript{2} can be increased by use of polar co-solvents, which enhance the solute solubility in SC-CO\textsubscript{2}.\textsuperscript{[13]} Thus in order to improve the yield under the optimized conditions of pressure and temperature, the effect of addition of co-solvents to SC-CO\textsubscript{2} on AX extraction yields was studied. In the present study, the solvents evaluated were methanol, ethanol and isopropyl alcohol. The correlation between solubility parameters of AX, CO\textsubscript{2} (300 bar) and entrainers (methanol, ethanol and 2-propanol) (300 bar) as a function of temperature is shown in Fig. 3. Ethanol at 20% v/w resulted in the highest AX recovery (963.33 μg/g) as compared to the other solvents. Ethanol at 20% v/w behaves as a potent chaotrope that can readily lyse all the cells of the ethanol-tolerant strain of \textit{Saccharomyces cerevisiae}.\textsuperscript{[3]} The presence of entrainers along with SC-CO\textsubscript{2} reduces the difference between SC-CO\textsubscript{2} (with entrainer) and AX. This enhances the solubility of AX in SC-CO\textsubscript{2}, leading to increased extraction. The control study with conventional extraction of AX\textsuperscript{[13]} without SC-CO\textsubscript{2} resulted in the extraction to be 317μg/g. Hence, the SC-CO\textsubscript{2} extraction under optimized conditions was found to be 203% higher than the conventional solvent extraction method.

Kagliwal et al.\textsuperscript{[25]} reported the use of 2-propanol as an entrainer at 30% v/w for the extraction of carotenoids from dried sea buckthorn seed powder. This enhanced the recovery of tocopherols and carotenes to 91.1% and 69.6% w/w, respectively. All the co-solvents except methanol at 10 and 20% v/w increased the extraction of AX (Fig. 4). The addition of co-solvents at 20% v/w increased the extraction of AX marginally from 834 to 860 μg/g. Ethanol is popularly recommended to be used as an effective co-solvent for AX extraction during SC-CO\textsubscript{2} extraction. The GRAS status of ethanol further makes it favourable for food applications.\textsuperscript{[36]} The increase in co-solvent concentration beyond 20% v/w did not have any additional effect on the extraction of AX.

SCFE has been used to extract carotenoids by various researchers.\textsuperscript{[15,16,27,28]} The solubility of targeted carotenoids in supercritical fluids depends not only on physical and chemical properties but also on the operating conditions such as temperature, pressure, density...
of solvent and solvent flow rate in the supercritical region.\(^{37}\) The use of co-solvent (methanol and ethanol) for extraction is also known to significantly increase the extraction yield. Mezzomo et al.\(^{16}\) compared the effect of hexane:isopropanol solution, 50:50, and sunflower oil as co-solvents at concentrations of 2 and 5% v/w to extract AX from pink shrimp (\textit{Penaeus brasilienis} and \textit{Penaeus paulensis}) processing waste. Sunflower oil showed a lower yield of carotenoid extraction but made the process selective for carotenoid extraction, while 2% hexane:isopropanol significantly increased the extraction yield.

### Mathematical modelling of AX extraction

The supercritical extraction of AX can be expressed as a function of pressure, temperature, particle size and entrainer (solvent) properties. The effect of solvent properties on the extraction efficiency of AX can be considered in terms of its solubility parameter. The solvents that exhibit greater affinity with AX are expected to have close solubility parameter values and vice versa.

\[
SE = f(P, T, d_p, \delta)
\]

where \(SE\) indicates supercritical extraction of AX in \(\mu\)g/g of solid while \(P, T, d_p\) and \(\delta\) are pressure, temperature, particle diameter and solubility parameter, respectively. The above equation can be transformed to the dimensionless form as follows:

\[
\overline{SE} = K \left( \frac{P}{P_c} \right)^a \left( \frac{T}{T_c} \right)^b \left( \frac{d_p}{d_{\text{pintact}}} \right)^c \left( \frac{\delta}{\delta_{\text{CO}_2}} \right)^d
\]

where \(\overline{SE}\) is the supercritical extraction of AX with respect to initial content of AX. \(P_c, T_c, d_{\text{pintact}}\) and \(\delta_{\text{CO}_2}\) are \(\text{CO}_2\) critical pressure, \(\text{CO}_2\) critical temperature, intact particle diameter and solubility parameter of \(\text{CO}_2\), respectively. The method of non-linear regression has been applied to evaluate the coefficient and exponents of each dimensionless term.\(^{38}\)

Figure 5 shows the parity plot. The statistical analysis of the data was also performed and statistical parameters such as correlation coefficient (\(R^2\)), Chi-square (\(\chi^2\)) and the root mean square error (RMSE) were estimated to assess the goodness of fit of the mathematical model to the experimental data. Generally, the higher \(R^2\) and the lower \(\chi^2\) and RMSE values give better model fitting. The Chi-square (\(\chi^2\)) and RMSE were calculated as follows:

\[
\chi^2 = \frac{\sum_i (SE_{\text{exp},i} - SE_{\text{pred},i})^2}{N - n}
\]

\[
\text{RMSE} = \left[ \frac{1}{N} \sum_i (SE_{\text{exp},i} - SE_{\text{pred},i})^2 \right]^{0.5}
\]

where \(SE_{\text{exp},i}\) and \(SE_{\text{pred},i}\) are the \(i\)th experimental and predicted supercritical extraction ratio, respectively. \(N\) is the number of observations and \(n\) is the number of parameters.

Table 3 shows the estimated values of model parameters, \(R^2\), \(\chi^2\) and RMSE. The \(R^2\), \(\chi^2\) and RMSE values indicate that mathematical model fits well to the experimental data. The proposed model, therefore, could be used to predict the extent of supercritical extraction of AX within the range of operating parameters studied in the present investigation.

![Figure 4. Effect of co-solvent on the extraction of AX. Empty bar, 10% v/w co-solvent; gradient fill bar, 20% v/w co-solvent.](image)

![Figure 5. Parity plot with standard deviation (±8%).](image)
The correlation obtained is as follows:

$$SE = 0.015 \left( \frac{P}{P_c} \right)^{2.75} \left( \frac{T}{T_c} \right)^{0.005} \left( \frac{d_p}{d_{p\text{intact}}} \right)^{-0.1} \left( \frac{\delta}{\delta_{CO_2}} \right)^{0.2}$$

(9)

Eq. (9) shows that operating pressure, particle size and solvent property have a significant effect on the extraction of AX while temperature has a negligible effect. Besides, the operating pressure has a positive effect on the extraction of AX while the reverse is true for particle size.

Conclusions
The solubility parameter approach gives an insight into the extraction behaviour of AX from Paracoccus MBIC 01143 as a function of pressure, temperature, particle size and extraction time. The operating conditions were optimized as a temperature of 40°C, pressure of 300 bar and extraction time of 60 min. Use of 2-propanol and ethanol as an entrainer at 20% v/w of dried biomass under the optimized conditions enhanced the extraction of AX. An increased recovery of 203% of AX was obtained after SC-CO$_2$ extraction in comparison with the conventional solvent extraction. Based on the experimental results, a correlation that satisfactorily fitted the experimental data could be developed. Additional scale-up trials at pilot plants would be required to use it effectively and efficiently at the industrial level.

Nomenclature

| d$_p$ | particle diameter, (mm) |
| E   | cohesive energy, (cal/mol) |
| H   | enthalpy, (cal/mol) |
| K   | coefficient defined in Eq. (6), (-) |
| n   | number of parameters |
| N   | number of observations |
| P   | pressure, (bar) |

Greek words

| $\rho$ | density, (gm/cm$^3$) |
| $\chi^2$ | Chi-square: statistical analysis parameter |

Subscript

| c  | critical condition |
| i  | special |
| L  | liquid state |
| r  | reduced condition |
| SF | supercritical fluid |
| vap | vaporization |

Funding
The authors gratefully acknowledge the UGC-SAP, Government of India, for their financial assistance during the course of this investigation.

References


