Optimal selectivity of γ-oryzanol and total phenolic compounds from rice bran using supercritical carbon dioxide fractionation technique


1Department of Food Science, National Chiayi University, 300 University Road, Chiayi City 60004, Taiwan
2Superwell Biotechnology Corporation, 465 Wenxin S. 2nd Road, Taichung City 40876, Taiwan

Abstract

The objective of the present work was to establish the optimal parameters for the selectivity of bioactive components; γ-oryzanol and total phenolic compounds, from ethanolic extract of rice bran using a continuous supercritical carbon dioxide (SC-CO₂) fluid fractionation technique. The ethanolic extracts of rice bran were separated into insoluble residue (R) and soluble fraction (F) according to an orthogonal design: temperatures (40, 45 and 50°C), pressures (20, 25 and 30 MPa), SC-CO₂ flow rate (2, 4 and 6 mL/min) and feed flow rate (1, 2, and 3 mL/min). Our data found that γ-oryzanol and total phenolic compounds were mainly partitioned in F and R, respectively. The optimal selectivity of γ-oryzanol and total phenolic compounds was obtained with the SC-CO₂ operation conditions of 50°C, 30 MPa, 6 mL/min SC-CO₂ flow rate, and 1 mL/min feed flow rate. Concurrently, a high regression coefficient via linear structural model analysis indicated that the response equation fitted well with the experimental data.

Introduction

The worldwide annual production of rice and rice bran, a by-product from rice milling, is 610 and 60 million tons, respectively (Sereewatthanawut et al., 2008; Pourali et al., 2009; Sookwong and Mahatheeranont, 2017). Rice bran contains many nutritious components including protein, fatty acids, dietary fibre, vitamins, minerals, and several bioactive compounds such as γ-oryzanol, tocopherols, tocolicrenols, phytic acid and phenolic compounds (Saenkod et al., 2013; Jun et al., 2015; Sookwong and Mahatheeranont, 2017).

Phenolic compounds and γ-oryzanol, the major antioxidants in rice bran (Ismail et al., 2010), have been reported to exert many beneficial effects such as nervous protection (Khanna et al., 2005), anti-hypercholesterolemic (Wilson et al., 2007), diabetes prevention (Chen and Cheng, 2006), prevention of thrombosis and arteriosclerosis, and anti-angiogenesis (Miyazawa et al., 2009; Sookwong and Mahatheeranont, 2017). γ-oryzanol, a phytosterol ferulate mixture, is composed of ferulic acid and triterpene alcohol. The main constitutes include cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate (Nystrom et al., 2005; Agarwal et al., 2016; Joshi et al., 2016).

Hexane is a commonly used solvent for the extraction of hydrophobic bioactive compounds from rice bran. After the removal of hexane, the residue is generally referred as rice bran oil (RBO) which contains γ-oryzanol (10-20 mg/g), tocotrienols (1.5-2 mg/g), and other impurities such as rice bran wax (2-4%), gums or phosphatides (1-2%), free fatty acids (5-25%) and colouring substances (Balachandran et al., 2008). Alternatively, supercritical carbon dioxide (SC-CO₂) fluid was successfully developed to extract oil, antioxidant, and bioactive compounds from various plants or herbs including Echinophora platyloba DC., Portulaca oleracea seed, and Ferulago angulata (Sodeifian et al., 2016; Sodeifian and Sajadian, 2017; Sodeifian et al., 2018). In the study of Sookwong and Mahatheeranont (2017), SC-CO₂ technique was applied to prepare RBO. RBO from SC-CO₂ contained more γ-oryzanol than RBO prepared with hexane. However, RBO from

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both extraction methods require a series of refining procedures including degumming, dewaxing, deacidification, bleaching and deodorisation to obtain substantially purified \( \gamma \)-oryzanol (Shukla and Pratap, 2017). Refining process usually results in significant loss of \( \gamma \)-oryzanol (Balachandran et al., 2008; Sookwong and Mahatheeranont, 2017).

Recently, a batch-type SC-CO\(_2\) fluid extractive fractionation technology conducted at different operating temperatures, pressure and flow rates was applied to separate active ingredients such as \( \gamma \)-oryzanol, tocopherols, tocotrienols and alkaloids of rice bran based on the solubility (Yoon et al., 2014; Khaw et al., 2017). Another rapid and continuous SC-CO\(_2\) study conducted by Fujii et al. (2018) showed the rapid extraction of vanillin via mixing of SC-CO\(_2\) and liquid solution to enhance the mass transfer. Moreover, we have also successfully enhanced and fractionated bioactive ingredients of several natural sources (propolis and Panax ginseng) in fractions with relative composition distribution and thus exhibited various biological properties in a continuous SC-CO\(_2\) fluid extractive fractionation system (Wang et al., 2004; Chien et al., 2016). For example, rich deglycosylated ginsenosides-fraction from ginseng markedly protected retinal pigment epithelium from \( \text{H}_2\text{O}_2 \)-induced oxidative damage (Yang et al., 2016) and flavonoids-rich fractions from propolis significantly suppressed \( \text{t-BHP} \)-induced liver damage (Wang et al., 2004). In the present work, rice bran was firstly extracted with ethanol to obtain hydrophilic components; and then the ethanolic extract was fractionated with supercritical \( \text{CO}_2 \) using multiple reactors in series to increase the purity of \( \gamma \)-oryzanol and total phenolic compounds. The objective of the present work was therefore to investigate the separation parameters for the optimal selectivity of \( \gamma \)-oryzanol and total phenolic compounds for further applications.

**Materials and methods**

**Ethanol extract of rice bran**

Rice bran powders (Rice Garden Milling Company, Chiayi, Taiwan) was sieved with a 0.42 mm screen and mixed with 95% ethanol at a ratio of 1:10 (w/v). The mixture was allowed to set for 24 h and filtered through Advantec filter paper No. 1. (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The ethanolic extract of rice bran (E) was stored at -20°C until use.

**Fractionation of \( \gamma \)-oryzanol and total phenolic compounds using SC-CO\(_2\)**

A schematic flow diagram of a continuous SC-CO\(_2\) fluid fractionation system is shown in Figure 1. Rice bran ethanolic extract (E) was separated into \( \gamma \)-oryzanol and total phenolic compound-rich fractions using a continuous supercritical carbon dioxide (SC-CO\(_2\)) fluid fractionation technique based on the method of Yu et al. (2016). Thermally labile compounds such as phenolic compounds would degrade when using extraction temperature up to 60°C (Sookwong and Mahatheeranont, 2017).

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**Figure 1.** A schematic flow diagram of a continuous SC-CO\(_2\) fractionation system. E: extractor; Feed: sample feed; HP1: super-critical CO\(_2\) pump; HP2: high-pressure liquid pump; TC: temperature gauge; P: pressure gauges; V1-V4: high-pressure valves.
While pressure is over 30 MPa, the density of CO$_2$ closely reaches steady state between the fluid and liquid phases. On the other hand, the relative ratio of SC-CO$_2$ flow rate and feed flow rates is designed to base on the steady state between the fluid and liquid phases (Rizvi et al., 1993; Yu et al., 2016; Fujii et al., 2018). Thus, an orthogonal design of the operating conditions: temperatures (40, 45 and 50°C), pressures (20, 25 and 30 MPa), SC-CO$_2$ flow rates (2, 4 and 6 mL/min) and feed flow rates (1, 2 and 3 mL/min) was performed (Table 1). The ethanolic extract and SC-CO$_2$ were continuously pumped into the continuous supercritical fluid system equipped with a 500 mL cylinder (316L-50DF4-500; Swagelok Co., Solon, OH, USA) packed with glass beads (3 mm, Kimax, Kimble, Vineland, NY, USA). The soluble and insoluble compounds of rice bran extracts in SC-CO$_2$ fluid were separated into the F fraction and the bottom residue (R) fraction, respectively. The collected samples were then stored at -20°C until use.

### Determination of γ-oryzanol content

The γ-oryzanol contents in E, F, and R fractions were analysed based on the HPLC method of Chen and Bergman (2005) with slight modifications. The HPLC (HPLC L-7100, Hitachi Ltd., Tokyo, Japan) system was equipped with a Hypersil ODS-5 C18 column (25 cm × 4.6 mm id) (Thermo Scientific Co., Waltham, MA, USA). The mobile phase consisted of acetonitrile, methanol and 0.03% acetic acid at a ratio of 45:52:3 (v/v), flow rate of 1 mL/min and detection wavelength of 300 nm.

### Determination of total phenolic compounds

The contents of total phenolic compounds in E, F, and R fractions were analysed spectrophotometrically using Folin–Ciocalteu’s reagent based on the method described by Wang et al. (2005). Sample (gallic acid as the standard or fractions, 0.1 mL), Folin-Ciocalteu’s reagent (0.5 mL, Sigma Chemical Co., St Louis, MO, USA), and sodium carbonate (7.5%, 0.4 mL) were thoroughly mixed. Following the incubation in the dark at room temperature for 40 min, the absorbance at 760 nm was measured. The content of total phenolic compounds was expressed as mg gallic acid equivalent/g dry weight of fraction (mg GAE/g dw).

### Selectivity of γ-oryzanol and total phenolic compounds

The selectivity, defined as the ratio of γ-oryzanol content in F to the content of total phenolic compounds in R, was used to evaluate the partition of these two type components in the F and R fractions based on the description of Yu et al. (2016). Following the fractionation of γ-oryzanol and total phenolic compounds using SC-CO$_2$, predicted selectivity was calculated using equation (1):

\[
\text{Predicted selectivity} = C1 \times \text{pressure} + C2 \times \text{temperature} + C3 \times \text{SC-CO}_2 \text{ flow rate} + C4 \times \text{feed flow rate}
\]

where C1, C2, C3 and C4 = linear regression coefficients.

### Analysis of DPPH radical scavenging capacity

The scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined based on the method of Shimada et al. (1992). Briefly, sample (extract or fractions, 0.25 mL) and DPPH solution (0.1 mM, 1.25 mL, Sigma Chemical Co., St Louis, MO, USA) were thoroughly mixed. The mixture was incubated in the dark at room temperature for 40 min, and then the absorbance at 517 nm was read. The DPPH radical scavenging capacity was calculated using equation (2):

\[
\text{DPPH scavenging capacity} (\%) = \left[1 - \frac{A}{A_0}\right] \times 100
\]

where $A_0$ = blank absorbance, and $A$ = sample absorbance.

### Analysis of ABTS$^+$ radical scavenging capacity

The total antioxidant activity was determined based on the method of Arnao et al. (2001) with slight modifications. Briefly, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 100 mol/L, 0.25 mL, Sigma Chemical Co., St Louis, MO, USA), peroxidase (4.4 U/mL, 0.25 mL), H$_2$O$_2$ (50 mol/L, 0.25 mL) and distilled water (1.5 mL) were mixed and kept in the dark for 1 h until the formation of stable blue-greenish ABTS$^+$. Sample (extract or fractions, 0.2 mL) was added and the absorbance at 734 nm was measured. The ABTS$^+$ scavenging capacity was calculated using equation (3):

\[
\text{ABTS}^+ \text{scavenging capacity} (\%) = \left[1 - \frac{A}{A_0}\right] \times 100
\]

Where $A_0$ = blank absorbance, and $A$ = sample absorbance.
Statistical analysis

One-way analysis of variance (ANOVA) with Duncan’s multiple range tests was conducted using SPSS for Windows software (SPSS, version 17.0; Technical Data and Computer Software, Chicago, IL., USA). Data were expressed as mean ± SD of triplicate determinations. The optimum response equation was obtained using multiple linear regression analysis. Linear structural models were conducted to find out the correlation between different operating conditions (pressure, temperatures, feed flow rate and SC-CO$_2$ flow rate) and selectivity using AMOS (Analysis of Moment Structure; SPSS Amos, version 21.0). A $p$-value of $<0.05$ was considered statistically significant.

Results and discussion

Table 1 lists the contents of γ-oryzanol and phenolic compounds in F and R fractions obtained at various SC-CO$_2$ operating conditions. The contents of γ-oryzanol and total phenolic compounds in the ethanolic extract (E) were 94.37 mg/g dw and 36.28 mg GAE/g dw, respectively. SC-CO$_2$ process significantly increased the level of γ-oryzanol in F fraction to 282.7-354.7 mg/g dw without affecting the content of total phenolic compounds in R (27.89-35.86 mg GAE/g dw).

Hexane or SC-CO$_2$ is generally used to extract γ-oryzanol from rice bran (Patel and Naik, 2004; Mariod et al., 2014; Shukla and Pratap, 2017). Compared to solvent extraction, SC-CO$_2$ possesses shorter extraction time and higher extraction efficiency (Bitencourt et al., 2016). In the report of Imsanguan et al. (2008), higher yield of γ-oryzanol in rice bran oil was extracted by SC-CO$_2$ as compared to n-hexane-Soxhlet due to the degradation resulting from harsh conditions of long time and high temperature. About 5.39 mg γ-oryzanol/g rice bran was obtained when extracted in a batch SC-CO$_2$ system at 68.9 MPa and 50°C; while 11.37 mg γ-oryzanol/g rice bran was obtained in a continuous SC-CO$_2$ system at 48 MPa and 65°C (Xu and Godber, 2000; Imsanguan et al., 2008). Similar to this continuous SC-CO$_2$ fractionation technique, ginsenosides and polysaccharides of ginseng (Yu et al., 2016), and tocotrienols and γ-oryzanol from rice bran oil (Khaw et al., 2017) were also successfully separated based on the material solubility. In the present work, the γ-oryzanol and phenolic compounds were unevenly distributed in different fractions, higher γ-oryzanol content was found in the F fraction, and higher phenolic content was found in the R fraction.

Selectivity was calculated as the ratio of γ-oryzanol content in F to phenolic contents in R. The experimental and predicted selectivity of γ-oryzanol to total phenolic compounds were 7.93-11.91 and 7.85-11.84, respectively (Table 1). The highest selectivity was obtained under the operating condition of 50°C, 25 MPa, 6 mL/min SC-CO$_2$ flow rate and 1 mL/min feed flow rate. The regressed coefficients between predicted selectivity and operating parameters from fitting experimental data into the equation were: $C_1 = 0.034, C_2 = 0.156, C_3 = 0.565, C_4 = -0.192$. A high $R^2$ (0.987) indicated that the response equation fitted well with the experimental data. Moreover, high correlations existed between selectivity and pressure (Pearson’s $r = 0.976, p < 0.01$), temperature (Pearson’s $r = 0.988, p < 0.01$), SC-CO$_2$ flow rate (Pearson’s $r = 0.949, p < 0.01$), and feed flow rate (Pearson’s $r = 0.907, p < 0.01$).

<table>
<thead>
<tr>
<th>Sample NO.</th>
<th>Pressure (MPa)</th>
<th>Temperature (°C)</th>
<th>SC-CO$_2$ flow rate (mL/min)</th>
<th>Feed flow rate (mL/min)</th>
<th>SC-CO$_2$ density (g/mL)</th>
<th>γ-oryzanol (mg/g dw)$^a$</th>
<th>Total phenolic compounds (mg/g dw)$^b$</th>
<th>Selectivity</th>
<th>Experimental</th>
<th>Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>40</td>
<td>2</td>
<td>1</td>
<td>0.84</td>
<td>347.6 ± 0.86$^b$</td>
<td>33.46 ± 2.64</td>
<td>10.43 ± 0.79$^c$</td>
<td>7.85</td>
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</tr>
<tr>
<td>2</td>
<td>25</td>
<td>40</td>
<td>4</td>
<td>2</td>
<td>0.88</td>
<td>287.9 ± 0.97$^c$</td>
<td>34.19 ± 4.18</td>
<td>8.51 ± 1.12$^d$</td>
<td>8.96</td>
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<tr>
<td>3</td>
<td>30</td>
<td>40</td>
<td>6</td>
<td>3</td>
<td>0.91</td>
<td>303.1 ± 0.65$^d$</td>
<td>27.89 ± 1.64</td>
<td>10.89 ± 0.63$^e$</td>
<td>10.06</td>
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</tr>
<tr>
<td>4</td>
<td>20</td>
<td>45</td>
<td>6</td>
<td>2</td>
<td>0.81</td>
<td>354.7 ± 0.05</td>
<td>34.28 ± 3.19</td>
<td>10.40 ± 0.98$^f$</td>
<td>10.70</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>45</td>
<td>2</td>
<td>3</td>
<td>0.86</td>
<td>299.6 ± 0.25$^f$</td>
<td>32.60 ± 2.73</td>
<td>9.23 ± 0.78$^g$</td>
<td>8.42</td>
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</tr>
<tr>
<td>6</td>
<td>30</td>
<td>45</td>
<td>4</td>
<td>1</td>
<td>0.89</td>
<td>336.2 ± 0.46$^g$</td>
<td>35.39 ± 1.93</td>
<td>9.52 ± 0.53$^h$</td>
<td>10.11</td>
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<tr>
<td>7</td>
<td>30</td>
<td>50</td>
<td>2</td>
<td>2</td>
<td>0.87</td>
<td>282.7 ± 0.41$^h$</td>
<td>35.70 ± 1.84</td>
<td>7.93 ± 0.48$^i$</td>
<td>9.56</td>
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<tr>
<td>8</td>
<td>20</td>
<td>50</td>
<td>4</td>
<td>3</td>
<td>0.79</td>
<td>335.1 ± 0.33$^i$</td>
<td>35.86 ± 3.75</td>
<td>9.42 ± 1.06$^j$</td>
<td>10.16</td>
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<td>25</td>
<td>50</td>
<td>6</td>
<td>1</td>
<td>0.83</td>
<td>347.0 ± 0.29$^j$</td>
<td>29.16 ± 1.07$^j$</td>
<td>11.91 ± 0.43</td>
<td>11.84</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Milligrams of γ-oryzanol/g of fraction dry weight.
$^b$Milligrams of gallic acid equivalents (GAE)/g of residual fraction dry weight.
Values in the same column with different superscripts are significantly different at $p < 0.05$. 
The predicted selectivity as a function of pressures and temperatures at various SC-CO₂ flow rates and at a constant feed flow rates of 1 mL/min is shown in Figure 2. The predicted selectivity increased with increasing SC-CO₂ flow rates, pressure and temperature at 1 mL/min feed flow rate. The optimum separation of γ-oryzanol and total phenolic compounds was found at 30 MPa, 50°C, and 6 mL/min SC-CO₂ flow rate. The optimal separation parameters of γ-oryzanol and phenolic compounds as a function of pressures and temperature at 2 mL/min SC-CO₂ flow rate and varying feed flow rates from 1 to 3 mL/min are shown in Figure 3. Operating pressures and temperatures significantly increased the selectivity of γ-oryzanol and phenolic compounds but feed flow rates decreased the separation efficacy of γ-oryzanol and phenolic compounds. The highest selectivity was obtained under the operating condition of 50°C, 30 MPa, and 1 mL/min feed flow rate. The predicted slope for a given selectivity of the SC-CO₂ flow rate (C₃), temperature (C₂) and pressure (C₁) parameter were 0.565, 0.156, and 0.034, respectively. SC-CO₂ flow rate mainly increased the selectivity under a constant operating temperature and pressure.

During SC-CO₂ extraction, pressure and temperature in the critical region are the key extraction efficiency parameters via regulating solute solubility and fluid diffusion to solutes (Khaw et al., 2017). SC-CO₂ density at high pressures usually increases solute solubility (Imsanguan et al., 2008; Belwal et al., 2016; Khaw et al., 2017), while temperature increases extraction yield by increasing solvent diffusion and decreasing viscosity (Imsanguan et al., 2008). However, in our observation at 20 MPa, the yields of γ-oryzanol decreased from 55.8% to 11.3% with the temperature increasing from 40°C to 80°C. Similar to previous reports (Machmudah et al., 2007; Tomita et al., 2014), lower solubility of γ-oryzanol in SC-CO₂ was found due to the temperature decreased the SC-CO₂ density. At pressure above 30 MPa, temperature assisted the solubility of γ-oryzanol. In the study of Bitencourt et al. (2016), a crossover pressure at 30 MPa was found. The solubility of γ-oryzanol decreased with increasing temperature below 30 MPa but increased with increasing temperature above 30 MPa. In this continuous SC-CO₂ system, pressure and temperature were the determinant factors for the separation of γ-oryzanol and phenolic compounds. The optimal separation of γ-oryzanol and phenolic compounds was achieved at 50°C, 30 MPa, SC-CO₂ flow rate of 6 mL/min and feed flow rate of 1 mL/min.

In the report of Khaw et al. (2017), operating temperature and pressure governed physical properties of the separator. Figure 2. Three-dimensional plot of predicted selectivity as a function of temperature and pressure. Feed flow rate was 1 mL/min and SC-CO₂ flow rates were: (A) 2 mL/min, (B) 4 mL/min and (C) 6 mL/min.
properties of SC-CO$_2$ fluid, including density, viscosity and diffusion coefficient. However, Ben-Rahal et al. (2015) found that increasing temperature decreased the yield of milk thistle seeds due to competition between solvent density and solute vapour pressure. Increasing in kinetic energy from temperature was directly proportional to diffusion rates of CO$_2$ within the raw plant material. Linear structural equation models such as Analysis of Moment Structure (AMOS) can be viewed as a combination of factor analysis and regression or path analysis (Mardani et al., 2017). In the present work, simple linear regression and correlation analysis between pairs of explanatory variables (operation temperature, pressure, SC-CO$_2$ density, SC-CO$_2$ flow rate and feed flow rate) and response variables (selectivity of γ-oryzanol and phenolic compounds) were analysed with AMOS based on the methods of Sheehan et al. (2000) and the results are shown in Figure 4.

Feed flow rate ($r = -0.14$, $p < 0.01$) was significantly negatively correlated to selectivity, while pressure ($r = 0.12$, $p < 0.01$), temperature ($r = 0.56$, $p < 0.01$) and SC-CO$_2$ flow rate ($r = 0.81$, $p < 0.01$) were significantly positively correlated to selectivity (Figure 4). The analysis of explanatory pairs (operation temperature, pressure, SC-CO$_2$ density, SC-CO$_2$ flow rate and feed flow rate) was not significantly correlated (Figure 4). In this linear structural equation model, the effects of SC-CO$_2$ flow rate on the selectivity of γ-oryzanol and phenolic compounds were more important than of pressure, temperature and feed flow rate.

DPPH$^+$ and ABTS$^-$ scavenging effects of different concentrations of E, R and F are shown in Figure 5. The scavenging capabilities of DPPH$^+$ (Figure 5A) and ABTS$^-$ (Figure 5B) increased with the concentration of rice bran extract or fractions. At 5 mg/mL, DPPH$^+$ scavenging abilities were in the order of E (81.03%) > F (77.56%) > R (63.13%). Although phenolic compounds were the major components of defatted rice bran response for the DPPH$^+$ scavenging activities, other components including proteins, carbohydrates and undetermined phenolic substances might also involve in DPPH scavenging (Renuka Devi and Arumughan, 2007). ABTS$^-$ scavenging activities were low and in the order of F (35.53%) > E (33.69%) > R (25.70%). The steric hindrance of hydrogen donation from the complex ester structure of γ-oryzanol might be the mechanism of low ABTS$^-$ scavenging activities (Saenjum et al., 2012).

Figure 3. Three-dimensional plot of predicted selectivity as a function of temperature and pressure. SC-CO$_2$ flow rate was 2 mL/min and feed flow rates were: (A) 1 mL/min, (B) 2 mL/min and (C) 3 mL/min.

Figure 4. Linear structural equation model of the effects of operation temperature, pressure, SC-CO$_2$ density, SC-CO$_2$ flow rate and feed flow rate on the selectivity of γ-oryzanol and phenolic compounds.
Figure 4. Linear structural models of determinants of selectivity as a function of temperature, pressure, SC-CO\textsubscript{2} density, SC-CO\textsubscript{2} flow rate and feed flow rate. Values shown are path coefficients: solid line, significant ($p < 0.01$); dashed line, non-significant. Negative values indicate inverse relationships.

Figure 5. Scavenging effect (%) of different concentrations of extract and fractions on DPPH (A) and ABTS\textsuperscript{+} (B) radicals. Data are mean ± standard deviation, $n = 3$.
Conclusion

This continuous SC-CO₂ fractionation technique successfully partitioned γ-oryzanol and total phenolic compounds in F and R, respectively. The optimal selectivity of γ-oryzanol and total phenolic compounds was obtained with the SC-CO₂ operation conditions of 50°C, 30 MPa, 6 mL/min SC-CO₂ flow rate, and 1 mL/min feed flow rate, and those experimental selectivity fitted well with the predicted data. Concurrently, linear structural models analysis showed that the effects of SC-CO₂ flow rate on the selectivity of γ-oryzanol and phenolic compounds were more important than those of pressure, temperature and feed flow rate.

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