Biodiesel production from *Fructus Schisandraceae* seed oil

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In the present study, we used *Fructus Schisandraceae* as a raw material to produce biodiesel. The seed oil yield of *Fructus Schisandraceae* was found to be 41.926±0.206%. In the seed oil, major fatty acids were linoleic acid (75.21%) and oleic acid (16.32%). For biodiesel transesterification, we found that immobilized lipase-base catalyzed transesterification using methanol and tert-butanol was the optimal method. In this method, the yield of biodiesel could reach 89.140±1.270%. Main components of the produced biodiesel were methyl palmitate, methyl oleate and methyl linoleate; methyl linoleate was the most abundant one. The physicochemical properties of the biodiesel met all the standards of biodiesel and the key technical indicators exceeded that of the number zero diesel oil. Our findings not only turn the excessive waste into raw materials for biodiesel, but also have broad application prospects in development of clean energy.

**Keywords:** ANL-CSAC, biodiesel, food security, *Fructus Schisandraceae*, seed oil

### Introduction

Biodiesel, as an element of renewable energy family, refers to a form of vegetable oil- or animal fat-based diesel fuel. It is typically made by chemically reacting lipids with an alcohol producing fatty acid esters. The major constituent parts are long-chain alkyl (methyl, ethyl or propyl) esters. Advantages are obvious in comparison to mineral diesel. For instance, biodiesel does not contain sulfur aromatic hydrocarbon and other toxic substances. It also has lower residual carbon content after combustion and better lubrication. Therefore, biodiesel used as heating oil satisfy the needs of today's social food security requirement avoiding petrodiesel contamination. Furthermore, biodiesel mixed together with petrodiesel in any ratio is now widely used in various fields as a fuel for vehicle, railway and aircraft, or a solvent to clean oil spills.1,2

In previous studies, a variety of vegetable oils and other herbal oil crops3-5, as well as some woody oil crops6,7, are potential candidates to produce biodiesel. However, raw material deficiency, long producing cycle and high cost retard its development. Until now, no research has been conducted to explore the possibility to produce biodiesel using seed oil of *Fructus Schisandraceae* [fruits of *Schisandra chinensis* (Turcz.) Baill. & *S. sphenanthera* Rehd. et Wils.]. *Fructus Schisandraceae* or Schisandra fruit is a traditional Chinese medicine widely cultivated in the Northeast region of China. The seeds of *Fructus Schisandraceae* mainly contain fatty oil and volatile gas, which have fatal toxic effect to mice. As a result, the seeds of *Fructus Schisandraceae* is usually disposed as waste. In this case, it is both environmentally friendly and cost effectively to use *Fructus Schisandraceae* seeds as raw material for biodiesel production.

### Materials and Methods

**Materials and Equipment**

*Fructus Schisandraceae* were picked from Erdao in Jilin province. It was identified as the *Schisandra chinensis* (Turcz.) Baill (specimen no. 00105293, S0014317) by comparison with the specimen database of Chinese Academy of Sciences, Beijing. ANL (5-amino-1-napthol) was purchased from Sigma. Tris-HCl was purchased from Beijing Dingguo Biotechnology Company. The active floridin was purchased from Nanjing Yadong Aotu Mining Company. Coconut active charcoal was purchased...
from Beijing Zhonghang Yuhong Environmental Protection Technology Company. Other reagents and solvents used in experiments were of analytical grade or reagent grade as appropriate. GC-MS 5973I was purchased from Agilent, USA.

**Extraction of Fructus Schisandrae Seed Oil**

The method of extraction of *Fructus Schisandrae* seed oil was developed in our laboratory. In order to avoid the effects of moisture content on the mechanical crushing, the seeds were pre-dried to constant weight at 60°C and grinded into powder. Petroleum ether was selected as extraction solvent. The powder was further extracted with petroleum ether at different times and different temperatures to determine the optimal conditions. Then all the solutions were further clarified by centrifugation and filtration to gain the seed oil.

**Transesterification**

**Acid-base Catalyzed Transesterification**

Acid-base catalyzed transesterification was performed with some modification as described previously. It was carried out in a reactor under the optimum conditions that were obtained by single factor experiments and response surface methodology. After the mixture statically stratified, the residual methanol fraction in the top layer was removed using rotary evaporator. The remaining fraction was washed with distilled water and then extracted with diethyl ether for four times. Using anhydrous sodium sulfate to dry the extract of diethyl ether and after vacuum distillation and decolouration, the biodiesel was obtained. The decolorized biodiesel was further purified using column chromatography on silica gel and eluted by petroleum ether, and after removing the petroleum ether using rotary evaporator at 60°C, the purified biodiesel was obtained.

**Immobilized Lipase-base Catalyzed Transesterification Using Methanol as Solution**

**Preparation of ANL-CSAC**—After washing with distilled water, the CSAC (coconut shell active charcoal) was activated at 400°C, treated by hydrochloric acid (pH 1), then phosphate buffer (pH 5) was added and kept for 3 h. Further, washed with boiling water to remove the extra phosphate buffer and then dried at 200°C for 6 h. The ANL was dissolved in phosphate buffer (pH 6) and centrifuged at 10 min at 8000 rpm. The supernatant was transferred into 100 mL triangular flask and added with the activated CSAC at a mass ratio of 1:35 (ANL to CSAC). The triangular flask was then sealed and kept in constant temperature incubator shaker at 180 rpm and 40°C for 5 h. The obtained ANL-CSAC was dried in freeze drying machine for 12 h and was kept under seal.

**Preparation of Biodiesel**—This transesterification was performed as described previously with some modification. The seed oil and methanol were added in triangular flask under the optimum conditions, which were optimized using single factor experiments and response surface experiment. The residual methanol fraction in the supernatant was removed using rotary evaporator, and the remaining fraction was washed with distilled water and extracted with diethyl ether. The extract of diethyl ether was washed with distilled water to neutral, dried using anhydrous sodium sulfate and the crude biodiesel was obtained after vacuum distillation. The crude biodiesel was purified using column chromatography on silica gel and eluted using petroleum ether.

**GC-MS method**

The fatty acid methyl esters (contents were determined by gas chromatography coupled with mass spectrometer (Flame Ionization Detector). The method was developed in our laboratory. Briefly, separation was performed on a capillary column HP-225 (30 m×0.25 mm×0.25 µm). For the seed oil, hydrogen was used as the carrier gas, temperature of vaporization chamber: 300°C, and temperature of detector: 270°C. The GC conditions were as follows: the initial column temperature was 100°C and it was kept for 3 min. Further, the column temperature was programmed from 100-160°C at the rate of increase 10°C/min, kept for 5 min; then it was programmed from 160-280°C at the rate of increase 20°C/min, kept for another 5 min. For the purified biodiesel, nitrogen was used as the carrier gas, temperature of vaporization chamber: 280°C, and temperature of detector: 270°C. The GC conditions were as follows: the initial column temperature was 140°C and it was kept for 3 min. The column
temperature was programmed from 140-220°C at the rate of increase 10°C/min, kept for 5 min, then was programmed from 220-260°C at the rate of increase 20°C/min, kept for 10 min.

**Results**

Using single factor experiment, we determined the optimal conditions: reaction time was 60 min; reaction temperature was 60°C. The yield of the *Fructus Schisandrae* seed oil was 41.926±0.206%. Using GC-MS, we analyzed the composition of the seed oil. The results are shown in Fig. 1. After the retrieval analysis, we determined the mass spectrum peak of palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), respectively (*Suppl Figs S1-S4). The area normalization method was used to calculate the relative content of fatty acids. The major fatty acids of the seed oil were linoleic acid (75.21 %) and oleic acid (16.32%), followed by palmitic acid (2.63%) and linolenic acid (0.7%). Unsaturated fatty acids were the major fatty acids, comprised of 92.23% of the total. Oil molecules used to prepare biodiesel should have a carbon chain length within the scope of 16 to 20 atoms. *Fructus Schisandrae* seed oil had mainly the straight chain fatty acid and could be used as the raw material for biodiesel production.

![Graph showing the analysis of Fructus Schisandrae seed oil using GC-MC](image)

The acid value of seed oil was 2.14 mg KOH/g, the saponification value was 171 mg KOH/g, and the average mol wt was 996.86. Although the acid value was higher than the value (≤0.25 mg KOH/g) in GB11765-2003, it was consistent with the requirement of GB 2716-2005 (4.0 mg KOH/g). Low saponification value shows that the saponification production was low in the ester exchange reaction. These physicochemical parameters provide information for structural stability and quality assessment. They indicated the capacity of *Fructus schisandrae* seed oil used as raw material to produce biodiesel.

In our study, using single factor experiment and response surface experiment, we optimized four factors of the diverse reaction systems including oil-to-organic solvent ratio, reaction time, reaction temperature and catalyst dosage (Table 1). Acid-base catalyzed transesterification was carried out under the optimum condition of catalyst H₂SO₄ (2.2% v/v) and oil-to-methanol ratio (1:6 m/m). The average yield of biodiesel was recorded as 95.198±0.685%.

Immobilized lipase-base catalyzed transesterification using methanol as a solution was carried out with an oil-to-methanol ratio (1:2.3). After addition of ANL-CSAC (24%), the yield of biodiesel was 61.568±1.252%. Immobilized lipase-base catalyzed transesterification using methanol and tert-butanol as solutions was carried out with an oil-to-methanol ratio (1:3.5). Tert-butanol was added in an oil-to-tert-butanol ratio (1:0.8). After the addition of ANL-CSAC (19.6%), the yield of biodiesel was recorded as 89.140±1.270% (Fig. 2). Then GC-MS was employed to analyze the biodiesel composition produced using three preparation methods, respectively (*Suppl Figs S5-S7). The area normalization method was used to calculate the relative content of the fatty acid methyl esters (Table 2). No significant difference was observed in the composition of biodiesel from three different preparation methods (Fig. 2).

Overall, acid-base catalyzed transesterification was...

<table>
<thead>
<tr>
<th>No.</th>
<th>Oil-to-organic solvent ratio (m/m)</th>
<th>Catalyst dosage (v/v)</th>
<th>Reaction time (h)</th>
<th>Reaction temperature (°C)</th>
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</thead>
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<tr>
<td>1*</td>
<td>Oil-to-methanol (1:6)</td>
<td>H₂SO₄ (2.2%)</td>
<td>2.5</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>Oil-to-methanol (1:2.3)</td>
<td>ANL-CSAC (24%)</td>
<td>16</td>
<td>37.5</td>
</tr>
<tr>
<td>3</td>
<td>Oil-to-methanol (1:3.5)</td>
<td>ANL-CSAC (19.6%)</td>
<td>11</td>
<td>37.5</td>
</tr>
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</table>

*1. Acid-base catalyzed transesterification
2. Immobilized lipase-base catalyzed transesterification using methanol as solution
3. Immobilized lipase-base catalyzed transesterification using methanol and tert-butanol alcohol as solution

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**Table 1 — The optimal conditions of methanol-to-oil ratio, reaction time, reaction temperature and catalyst dosage in the reaction system**
the most efficient one. The yield of biodiesel using immobilized lipase-base catalyzed transesterification with methanol as solution was the lowest. However, the addition of tert-butanol improved the production. In addition, ANL-CSAC recycled after 20 times could still reach 50% biodiesel ester exchange rate (data not shown), this greatly reduced the cost. Thus, we chose immobilized lipase-base catalyzed transesterification using methanol and tert-butanol as the best solution to prepare biodiesel. In this case, the yield of biodiesel was comparable to the acid-base catalyzed transesterification. Moreover, this method has more simplified operation steps.

The produced biodiesel was tested in Chemical Product Supervision and Inspection Station of Jilin Province. The test results showed the following: cetane number 51, density (20°C) 0.868 g/cm³, kinematic viscosity (20°C) 4.2 mm²/s, flash point 137°C, sulfur content 3 mg/kg, no mechanical impurities, copper corrosion (50°C) 1, acid value 0.154 mg KOH/g, condensation point – 4°C and total glycerin content 0.21%. All these values were in accordance with the standard of biodiesel and the key technical indicators exceeded that of the number zero diesel.

**Discussion**

In the present study, we used *Fructus Schisandrae* as a raw material to produce biodiesel. Three transesterification methods were performed. Although acid-base catalyzed transesterification was the most efficient one, but considering the residual byproduct as acid, which would cause the corrosion to the metal and an additional decolorization step was needed, this method was not ideal. In biodiesel preparation, catalysts were added in order to achieve higher conversion rate. The characteristic of a catalyst is very critical in biodiesel production. It regulates the recipe of raw materials, reaction conditions and post-processing steps. Instead of any pretreatment steps, it is much more effective to use acid-catalyzed reaction to eliminate free fatty acid and save raw materials. At the production level, enzyme catalysts out performed in many ways including mild reaction conditions, easier separation procedures and no pollutant byproduct. However, the cost of enzyme mediated biodiesel production is relatively high due to poor stability of lipase and its activities can easily be affected or inactivated by external factors. Immobilized lipase is another promised industrial catalyst for transesterification reaction with high stability, good separability and also reusability. Although immobilized lipase has many advantages as the catalyst in biodiesel production, the enzyme activity is compromised in the presence of methanol, which may lead to decreased biodiesel yield. It is documented that adding organic solvent can enhance the miscibility of methanol and waste oil, while reducing the viscosity of the reaction mixture at the

![Fig. 2 — The yield of biodiesel produced by three transesterification technologies and comparison of their composition. [A, Acid-base catalyzed transesterification; B, Immobilized lipase-base catalyzed transesterification using methanol as a solution; C, Immobilized lipase-base catalyzed transesterification using methanol and tert-butyl alcohol as a solution.]](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Fatty acid methyl esters</th>
<th>Formula</th>
<th>A*</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl palmitate</td>
<td>C₁₇H₃₄O₂</td>
<td>2.77</td>
<td>2.75</td>
<td>2.84</td>
</tr>
<tr>
<td>2</td>
<td>Methyl oleate</td>
<td>C₁₉H₃₈O₂</td>
<td>17.43</td>
<td>17.73</td>
<td>17.29</td>
</tr>
<tr>
<td>3</td>
<td>Methyl linoleate</td>
<td>C₁₉H₃₈O₂</td>
<td>73.78</td>
<td>74.58</td>
<td>74.66</td>
</tr>
<tr>
<td>4</td>
<td>Others</td>
<td></td>
<td>6.02</td>
<td>4.94</td>
<td>5.21</td>
</tr>
</tbody>
</table>

*A. Acid-base catalyzed transesterification
B. Immobilized lipase-base catalyzed transesterification using methanol as solution
C. Immobilized lipase-base catalyzed transesterification using methanol and tert-butyl alcohol as solution*
same time. Meanwhile, the organic solvent added to the reaction system also act as a buffer to reduce the toxic effects of methanol to catalyst so that the biodiesel production yield can be increased$^{11,12}$. Our results are consistent with these reports. We demonstrated that the immobilized lipase-base catalyzed transesterification using methanol and tert-butanol was the most optimal method for the biodiesel transesterification. The yield of such biodiesel was 89.140±1.270%. The main components of the produced biodiesel were methyl palmitate, methyl oleate and methyl linoleate, with methyl linoleate as the most abundant one. Our findings not only turn the excessive waste into raw materials for biodiesel but also have broad application prospects in development of clean energy.

References