A New Homogenizing Technology to Obtain Rosmarinic Acid from Perilla Oil Meal

TANG Wei-zhuo1, LIU Yan-ze2,3, ZHAO Yu-qing1,4*

1. Shenyang Pharmaceutical University, Shenyang 110016, China
2. Bio-Organic and Natural Product Laboratory, MRC 311, McLean Hospital/Harvard Medical School, MA 02478, USA
3. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193
4. Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract: Objective To optimize the extraction technology of the active component, rosmarinic acid, an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, in perilla oil meal for the first time by a new homogenizing technology called smashing tissue extraction (STE). Methods Orthogonal design was used to optimize the extraction condition. The content of rosmarinic acid was quantified from the methanol crude extract with the help of HPLC. Results The optimization of STE process to get rosmarinic acid from the perilla oil meal was the ratio of liquid to solid material at 10:1 and the power of extraction at 150 V, extracting twice (2 min for each time). Conclusion STE could be applied to extracting the active ingredients from the oil meals due to its high extraction efficiency. This new homogenizing technology has advantages on saving extraction time, raising extraction efficiency, and maintaining the temperature sensitive constituents.

Key words: HPLC; perilla oil meal; rosmarinic acid; smashing tissue extraction; tissue homogenizing technology

Introduction

*Corresponding author: Zhao YQ  Address: College of Traditional Chinese Metaria Medica, Shenyang Pharmaceutical University, No. 103, Wenhua Road, Shenhe District, Shenyang 110006, China  Tel: +86-24-2398 6523  Fax: +86-24-2398 6522  E-mail: zyq4885@126.com

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Fig. 1 Chemical structure of RoA

(Lamien-Meda, Nell, and Lohwasser, 2010), antiviral (Dubois et al, 2008), anti-inflammatory (Jiang, Chen, and Qu, 2009), anticanecer (Scheckel, Degner, and Romagnolo, 2008) and anti-allergic (Lee, Jung, and Koh, 2008) activities. Hence, the study on isolating and obtaining of RoA is meaningful and valuable.

Extraction is a very fundamental and necessary step to use crude drug, whatever in manufacturing formulated patent medicines or preparing any single herbal extract. Traditionally, the major techniques to obtain RoA include Soxhlet extraction (Zhou and Lv, 2006; Luo et al, 2005), ultrasonic extraction (Zhou, Lv, and Yao, 2007; Ling, Zhai, and Li, 2008), SFE-CO2
(Chen et al., 2008), and other conventional extraction methods (Lv et al., 2010). The main disadvantages of those technologies are increased solvent cost and the potential to contaminate the environment with more solvent residue.

However, smashing tissue extraction (STE), a newly developed tissue homogenizer extraction technology, could significantly improve the efficiency to shorten the extraction time from minutes to seconds and has achieved quite success in several herbal cases (Liu et al., 2011; Sun et al., 2011). What’s more, another remarkable advantage of STE is the operation condition under room temperature. So, further investigation of STE for different herbal medicines containing different types of active constituents is very interesting and attractive.

In present study, STE was applied to extracting RoA from perilla oil meal and orthogonal design was employed in optimizing the extraction process.

**Materials and methods**

**Materials and reagents**

Samples of the perilla oil meal were obtained from Jiashi Healthcare Food Co., Ltd. (Shenyang, China). Methanol (HPLC and analytical grade), formic acid (analytical grade), and orthophosphoric acid (analytical grade) were purchased from Concord Chemical Agent Factory (Tianjin, China) and Shenyang No. 5 Chemical Agent Factory (Shenyang, China). The reference substance of rosmarinic acid was purchased from Jianfeng Natural Products Research Co., Ltd. with purity > 98.0% (Tianjin, China). The water used in HPLC and for sample preparation was obtained from Wahaha Group Co., Ltd.

**Equipments**

JHBE—50S Herbal Blitzkrieg Extractor (Henan Jinnai Sci-Tech Development Co., Ltd.); BS—124S precise electronic balance (Sartorius Corporation); RE—52A Rotary Evaporator (Shanghai Yarong-shenghua Instrument Factory); Kromasil C18 (250 mm × 4.6 mm, 5 μm) column; Beijing Chuangxintongheng high performance liquid chromatograph comprising a UV3000 ultraviolet detector, attemperator and CXTH—3000 work station.

**Preparation of reference solution**

RoA reference substance (2 mg), weighed accurately, was put into a separate 10 mL volumetric flask and dissolved in methanol with the aid of sonication.

**Sample preparation**

Perilla oil meal (10 g) was put into the STE cask, followed by defatting with the petroleum ether, and extracted with 100 mL of methanol for 2 min at 150 V with Smashing Tissue Extractor. Then the resulting mixture was centrifuged at 3500 r/min for 5 min and the supernatant was put into a 25 mL volumetric flask and dissolved in methanol for HPLC analysis followed by being transferred to a 250 mL round bottom flask for concentration.

**Chromatographic condition**

The separation system consisted of a C18 reversed-phase column, a gradient elution system of methanol-water containing orthophosphoric acid, and a UV detector. The column, a Kromasil C18 (250 mm × 4.6 mm, 5 μm) was maintained at 30 °C. The analytes were eluted at a flow rate of 1.0 mL/min using 0.05% orthophosphoric acid in water (A) and methanol (B). The linear gradient program was: 0—20 min, maintained at 40% B; 20—30 min, linear gradient from 40% to 35% B. Detection wavelength was 330 nm and the sample injection volume was 10 μL.

**Results**

**Optimum condition of STE**

The orthogonal test of three factors and three levels, i.e. liquid/solid ratio (A), STE time (B), and extraction power (C) was designed to find the optimum extraction condition of the active component, rosmarinic acid. Tables 1—3 show the process of the orthogonal test.

<table>
<thead>
<tr>
<th>Levels</th>
<th>Liquid/solid ratio</th>
<th>STE time / min</th>
<th>Extraction power / V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8:1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>10:1</td>
<td>1.5</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>15:1</td>
<td>2</td>
<td>150</td>
</tr>
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</table>

From these tables, the optimum STE conditions for extraction of rosmarinic acid from perilla and linseed oil cakes are obtained as follows: the liquid/solid ratio is 10:1, the STE time is 2 min, and the extraction power is 150 V.

**Optimum separation of RoA**

Organic acids including phosphoric acid and formic acid are usually added into the mobile phase to
Table 2  Results of L₉(3⁴) orthogonal test

<table>
<thead>
<tr>
<th>No.</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>RoA / (mg·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.9752</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1.042</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1.918</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1.350</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1.815</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1.520</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1.438</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1.244</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1.327</td>
</tr>
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<td></td>
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</tr>
</tbody>
</table>

\[ K_1 = 3.935 \quad 3.763 \quad 3.739 \quad 4.117 \]
\[ K_2 = 4.685 \quad 4.101 \quad 3.719 \quad 4.000 \]
\[ K_3 = 4.009 \quad 4.765 \quad 5.171 \quad 4.512 \]
\[ k_1 = 1.312 \quad 1.254 \quad 1.246 \quad 1.372 \]
\[ k_2 = 1.562 \quad 1.367 \quad 1.240 \quad 1.333 \]
\[ k_3 = 1.336 \quad 1.588 \quad 1.724 \quad 1.504 \]
\[ R = 0.250 \quad 0.334 \quad 0.484 \quad 0.171 \]

Table 3  Analysis of variance

<table>
<thead>
<tr>
<th>Factors</th>
<th>SS</th>
<th>N</th>
<th>F ratio</th>
<th>Critical value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.114</td>
<td>2</td>
<td>0.572</td>
<td>4.460</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.173</td>
<td>2</td>
<td>0.868</td>
<td>4.460 ( P &lt; 0.05 )</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.462</td>
<td>2</td>
<td>2.319</td>
<td>4.460 ( P &lt; 0.05 )</td>
<td></td>
</tr>
<tr>
<td>error</td>
<td>0.80</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In our previous work, STE had successfully been applied to extracting different kinds of active ingredients from the traditional Chinese medicinal herbs such as Panax notoginseng (Burkill.) Hoo et Tseng, Centella asiatica (Linn.) Urban., Momordica charantia (Linn.), and Glycyrrhiza glabra (Linn.). Results from these studies demonstrated that STE was significantly superior to other traditional techniques including lixiviation extraction, soxhlet extraction, and ultrasonic assistant extraction due to its ability to save both time and solvent volume. Simultaneously, this newly developed tissue homogenizer technology has been employed in current Chinese Pharmacopeia for quality control.

What’s more, we firstly focus on obtaining the natural phenolic acid, which makes a considerable contribution to the nutritional quality and plays an important role in the daily diet, from the residues of perilla seed oil. It brings a new source to get this active compound and provides another way to make good use of this scrap material. Meanwhile, the result has proved that STE combining smash, soak, stir, and vibration together is an effective and practicable extraction method named STE.
technology.

Therefore, it is really valuable for the utilization and exploitation of these byproducts of the vegetable oil processing industry. And also a new extraction technology could contribute to deeply understand the known active compounds and to discover more unknown active ingredients.

References


Sun Yan-ling, Liu Yan-ze, Xiao Han, Wei Ying-feng, Zhao Yu-qing, 2011. Smashing tissue extraction and GC analysis of active fatty acids from oil cake of perilla seeds. Chin Herb Med 3(1): 75-78.

