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

TANG Wei-zhuo[1], LIU Yan-ze[2, 3], ZHAO Yu-qing[1, 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
DOI: 10.3969/j.issn.1674-6384.2012.01.011

## Introduction

*Perilla frutescens* (Linn.) Britt, a herb belongs to the family of Labiatae, is an edible plant used as one of the most popular garnishes and food colorants in some Asian countries (Peng, Ye, and Kong, 2005). The perilla oil meal is defined as ground residue after the oil is removed from the perilla seeds with a high content of oil.

Rosmarinic acid (RoA) is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. It is commonly found in the species of Boraginaceae, Cucurbitaceae, Sterculiaceae, and the subfamily of Lamiaceae. It is also found in the species of other higher plant families, such as some fern and hornwort species. Rosmarinic acid was first isolated by two Italian chemists, Scarpati and Oriente, as a pure compound from *Rosmarinus officinalis* L. (Maike and Monique, 2002) and its structure was elucidated in Fig. 1.

RoA is reported to exhibit a number of interesting biological activities, such as high anti-oxidative (Lamien-Meda, Nell, and Lohwasser, 2010), antiviral (Dubois *et al*, 2008), anti-inflammatory (Jiang, Chen, and Qu, 2009), anticancer (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.

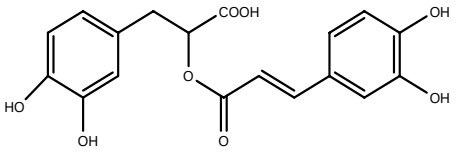


**Fig. 1 Chemical structure of RoA**

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-$CO_2$

* 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
Received: April 28, 2011; Revised: September 13, 2011; Accepted: October 9, 2011
Fund: E&T Modern Center for Natural Products of Liaoning Province of China (2008402021)

(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 attractable.

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 $C_{18}$ (250 mm × 4.6 mm, 5 μm) column; Beijing Chuangxintonghen 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 $C_{18}$ reversed-phase column, a gradient elution system of methanol-water containing orthophosphoric acid, and a UV detector. The column, a Kromasil $C_{18}$ (250 mm × 4.6 mm, 5 μm) was maintained at 30 ℃. 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 1 Factors and levels**

| Levels | Liquid/solid ratio | STE time / min | Extraction power / V |
|---|---|---|---|
| 1 | 8:1 | 1 | 100 |
| 2 | 10:1 | 1.5 | 120 |
| 3 | 15:1 | 2 | 150 |

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 $L_9(3^4)$ orthogonal test**

| No. | A | B | C | D | RoA / (mg·g$^{-1}$) |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 0.9752 |
| 2 | 1 | 2 | 2 | 2 | 1.042 |
| 3 | 1 | 3 | 3 | 3 | 1.918 |
| 4 | 2 | 1 | 2 | 3 | 1.350 |
| 5 | 2 | 2 | 3 | 1 | 1.815 |
| 6 | 2 | 3 | 1 | 2 | 1.520 |
| 7 | 3 | 1 | 3 | 2 | 1.438 |
| 8 | 3 | 2 | 1 | 3 | 1.244 |
| 9 | 3 | 3 | 2 | 1 | 1.327 |
| $K_1$ | 3.935 | 3.763 | 3.739 | 4.117 | |
| $K_2$ | 4.685 | 4.101 | 3.719 | 4.000 | |
| $K_3$ | 4.009 | 4.765 | 5.171 | 4.512 | |
| $k_1$ | 1.312 | 1.254 | 1.246 | 1.372 | |
| $k_2$ | 1.562 | 1.367 | 1.240 | 1.333 | |
| $k_3$ | 1.336 | 1.588 | 1.724 | 1.504 | |
| $R$ | 0.250 | 0.334 | 0.484 | 0.171 | |

**Table 3 Analysis of variance**

| Factors | SS | $N$ | $F$ ratio | Critical value | Significance |
|---|---|---|---|---|---|
| A | 0.114 | 2 | 0.572 | 4.460 | |
| B | 0.173 | 2 | 0.868 | 4.460 | $P < 0.05$ |
| C | 0.462 | 2 | 2.319 | 4.460 | $P < 0.05$ |
| error | 0.80 | 8 | | | |

get the best chromatographic peak when the phenolic acid is analyzed during the process of aromatic plants (Wang, Provan, and Helliwell, 2004; Abdullah, Schneider, and Petersen, 2008; Geller *et al*, 2010). In this paper, several mobile phases including methanol-water in combination with formic acid or phosphoric acid with different solvent ratios such as 40:60 were tested to get the best separation of RoA. Fig. 2 shows the different chromatograph conditions used to test the resolution of RoA. It could be seen from Fig. 2 that a water-methanol system containing 0.05% of phosphoric acid with a linear gradient program gave the best result and a good resolution of RoA in the perilla oil meal could be achieved within 30 min using the conditions described above.

In this condition, the content of RoA in perilla oil meal is 0.27% which is nearly to the content in stems and leaves of some aromatic herbs such as rosmary, perilla, lavender, peppermint, and *Prunella vulgaris* Linn. by using the new tissue homogenizer extraction method named STE.

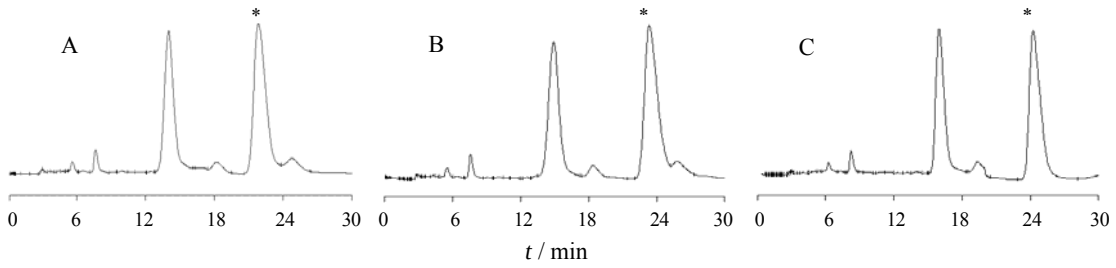


**Fig. 2 Chromatograms of different mobile phases tested for perilla oil meal**

A: 0.1% formic acid added in water

B: mobile phase at ratio of 40:60 with 0.05% phosphoric acid

C: linear gradient described above

*-RoA

## 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

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.

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