Microwave-assisted extraction of flavonoids from *Radix Astragali*

Weihua Xiao\textsuperscript{a}, Lujia Han\textsuperscript{a,\textdagger}, Bo Shi\textsuperscript{b}

\textsuperscript{a} Engineering College, China Agricultural University, Beijing 100083, PR China
\textsuperscript{b} Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China

\textbf{A R T I C L E   I N F O}

Article history:
Received 27 May 2007
Received in revised form 14 March 2008
Accepted 19 March 2008

Keywords:
Microwave-assisted extraction
Flavonoids
Radix Astragali
Stability

\textbf{A B S T R A C T}

Microwave-assisted extraction (MAE) technique was developed for the fast extraction of flavonoids from *Radix Astragali*. Several influential parameters of the MAE procedure (microwave power, extraction cycles, ethanol concentration, extraction temperature, irradiation time, and solvent to material ratio) were studied for the optimization of the extraction protocol. The maximum yield of flavonoids with MAE was obtained by dual extraction with 90% ethanol 25 ml/g material at 110 °C for 25 min. No degradation of the flavonoids was observed using the developed extraction protocol. The optimal yield with MAE 1.190 ± 0.042 mg/g were close to that of Soxhlet extracted with methanol for 4 h (1.292 ± 0.033 mg/g) and higher than that of ultrasound assisted extraction with methanol for 2 × 30 min and heat reflux extraction with 90% ethanol for 2 × 2 h.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

*Radix Astragali* (root of *Astragalus*; Huangqi) is one of the best-known natural traditional Chinese medical herbs along with Ginseng and Notoginseng. The main active constituents of *Radix Astragali* include flavonoids, saponins, polysaccharides, amino acids, and trace elements. Recent studies reveal that the flavonoids of *Radix Astragali* show strong antioxidant activity [1–3] and pharmacological properties such as effect on impairment of barrier function induced by hypoxia [4]. *Astragalus mongolicus* is the commonly used species and the main flavonoid contents are calycosin, formononetin, calycosin-7-O-β-d-glucoside and ononion [5].

Several techniques are available for the extraction of flavonoids from *Radix Astragali* including Soxhlet [6,7], ultrasound-assisted extraction (UAE) [8], and heat reflux extraction (HRE) [9]. Methanol was used as extraction solvent in the Soxhlet and ultrasound-assisted extraction methods, which was hazardous both to the operators and to the environment. HRE with ethanol is now in general practice applied for the scale-up industrial production in spite of it being time-consuming and labour intensive.

Many reports have been published on the application of microwave-assisted extraction (MAE) of secondary metabolites from plants [10–17]. Nevertheless, no reports on MAE of flavonoids from *Radix Astragali* have been published. The main advantages of MAE are the considerable reduction in time and solvent as compared to conventional techniques. In the paper we report on the feasibility of MAE for the rapid extraction of flavonoids from *A. mongolicus* and compare MAE with traditional techniques.

2. Materials and methods

2.1. Plant material, standards and reagents

*A. mongolicus* were bought from Beijing Tong Ren Tang Group Co., Ltd., in June 2006. The cut pieces were ground with a blade-mill (FW135 medicine mill, PR China) to obtain a relatively homogenous drug powder and then sieved through 10-mesh screen. The powder was dried at 60 °C until constant weight and was well blended before use.

All analytical grade solvents were from Beijing Chemical Plant (Beijing, PR China) and HPLC grade chemicals were from Fisher. Standard of calycosin (>90%) was obtained from the Shanghai R&D Center for Standardization of Chinese Medicine, calycosin-7-O-β-d-glucoside (>98%) from National Natural Product Standard Laboratory, and formononetin, and ononion (>98%) from Chromadex (Santa Ana, CA, USA).

2.2. Methods

2.2.1. Extraction methods

MAE was performed on microwave apparatus using closed-vessel system with pressure (ETHOS® T Microwave digestion/extraction system, Milestone Co., Italy). Several grams of drug powder varied according to the solvent to material ratio were put into a 100 ml PTFE extraction vessel extracted with...
50 ml solvent under different MAE conditions. After extraction, the vessels were allowed to cool at room temperature before opening. Microwave power (200–1000 W), number of extraction cycles (1–3 times), ethanol concentration (60–100%, v/v), temperature (70–130 °C), irradiation time (5–30 min) and ratio of solvent to material (10–40 ml/g) were evaluated for the extraction of flavonoids from *Radix Astragali*.

Soxhlet extraction was performed in a Soxhlet apparatus. Exhaustive extraction with methanol (85 °C) was performed on 4.0 g drug powder, placed in an extraction bag filter, and impregnated with methanol. Extraction was performed for about 4 h with 100 ml methanol.

HRE was conducted in a water bath at 75 °C. An amount of 10.0 g drug powder were placed into a 500 ml glass flask with 250 ml 90% (v/v) aqueous ethanol and extracted for two 2 h cycles.

Ultrasonic-assisted extraction was conducted in an ultrasonic bath (Shumei® KQ-800TDV ultrasonic instrument, Kunshan, PR China). Drug powder weighing 5.0 g were placed into a 250 ml volumetric flask with 100 ml methanol and sonicated in a water bath at 60 °C for two 30 min cycles.

All of the obtained solvent was then evaporated to dry, dissolved in methanol for HPLC analysis.

### 2.2.2. Stability of flavonoid during MAE

In order to evaluate the performance of different extraction conditions with accuracy, stability of *Radix flavonoid* during the extraction was determined prior to the method development. The stability study was performed using the extract obtained by HRE with 90% (v/v) aqueous ethanol as listed previously. The extract was centrifuged for 10 min, filtered through filter paper and stored at −18 °C. The concentration (mg L⁻¹) of calycosin, formononetin, calycosin-7-O-β-D-glucoside and ononion were 87.12, 22.37, 13.05, and 5.32, respectively.

When using high temperatures it is advisable to access the stability of target compounds. The selected temperature range is between 70 and 150 °C and extraction time should be below 30 min when handling a large number of samples. Therefore, the first aspect to be evaluated was the flavonoid stability under extraction conditions for 30 min using different temperatures. This will allow the selection of an adequate extraction temperature for an analytical method that improves extraction efficiency without affecting the flavonoid profile on the sample and a reasonable industrial extraction method without changing the main components of the materials.

### 2.2.3. HPLC analysis

Calycosin, formononetin, calycosin-7-O-β-D-glucoside and ononion, the four main flavonoids in *Radix Astragali* were quantified by high performance liquid chromatography–UV–vis detection (HPLC–UV) in our work. A Hitachi system (Japan) consisting of two MODEL L-7100 pumps, a MODEL L-7200 auto-sampler and a MODEL L-7420 detector were used. Experimental data were acquired and processed by Weimalong Software (Nanning, PR China).

Chromatographic separations were carried out using a Supelocosil™-C18 column (250 mm × 4.6 mm, 5 μm, Supelco USA) with a Pelliguard modular guard column LC-18 (250 mm × 4.6 mm, 5 μm, Supelco, USA). A gradient elution regime was employed using water (eluent A) and MeCN (eluent B). The composition of the eluent was varied from 0% to 28% B in 15 min, while the flow rate was changed from 1.2 to 1.0 ml/min; 28–38% B from 15 to 30 min and 38% B from 30 to 45 min, the flow rate was 1.0 ml/min. Column temperature was kept at 40 °C. UV detection: 230 nm.

All methanol mixtures obtained were centrifuged for 10 min at 3000 rpm and were filtered through 0.45 μm membrane. The injection volume was 10 μL. Each determination was carried out in duplicate. Quantitative determination of the considered phytochemicals in the extracts was performed using external standards by means of a six points calibration curve. The regression equations, correlation coefficient and linear range are listed in Table 1.

### 3. Results and discussion

#### 3.1. Flavonoid stability

The individual relative concentration of flavonoid in the extract (obtained previously by HRE) submitted to different temperatures under extraction conditions for 30 min is shown in Fig. 1. The values are relative to the initial flavonoid concentration in the HRE extract (100%). Extraction temperature has a clear effect on flavonoid concentration and can be divided in ranges. Extractions performed between 70 to 110 °C did not decrease flavonoid on the extract, whilst extractions performed at 130 °C expose Cg and C to

### Table 1

**Standard curve of flavonoids**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Symbol</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Linear range (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calycosin</td>
<td>C</td>
<td>y=4.45×10⁻⁵x+0.211</td>
<td>0.999</td>
<td>15–120</td>
</tr>
<tr>
<td>Formononetin</td>
<td>F</td>
<td>y=2.92×10⁻⁵x+0.025</td>
<td>0.998</td>
<td>5.5–44</td>
</tr>
<tr>
<td>Calycosin-7-O-β-D-glucoside</td>
<td>Cg</td>
<td>y=3.90×10⁻⁵x+4.57</td>
<td>0.999</td>
<td>35–280</td>
</tr>
<tr>
<td>Ononion</td>
<td>O</td>
<td>y=3.31×10⁻⁵x+1.75</td>
<td>1.000</td>
<td>28–224</td>
</tr>
</tbody>
</table>

C Calycosin-7-O-β-D-glucoside.

![Fig. 1. Stability of flavonoids during extraction at different temperatures. Extraction conditions: 10 ml of sample, 1000 W, 30 min.](image_url)
degradation and extraction at 150 °C cause the sharp degradation of Cg, C, and O. Concentration of F increased with the extraction temperature increasing from 70 to 150 °C. The degree of degradation at 150 °C increased with the molecular polarity increasing (F < C < O < Cg) for the microwave selective heating of polar molecular. F, with the weakest polarity among the four flavonoids, increased for the transforming of other derivatives at high temperature.

From the results we can infer that 70–10 °C is a safe temperature used for the development of a reliable extraction method.

3.2. Effect of microwave power

An amount of 5.0 g was extracted with 50 ml 90% aqueous ethanol at 90 °C for two cycles under different microwave powers (200, 400, 600, 800, and 1000 W) (see Fig. 2). In general, the extraction efficiency was improved by raising microwave power from 200 to 1000 W. During short irradiation time (5 and 10 min) yield of flavonoids were enhanced with microwave power increasing. When the extraction solutions were heated long enough (15 min), the yields under different powers were similar. The difference of the flavonoids yield among 200 to 1000 W appears more significant with short irradiation times compared to long irradiation times. A similar result was also reported previously in MAE of notoginseng saponins from cultured cells of *Panax notoginseng* [12] and MAE of flavonoids from *Saussurea medusa* maxim cultured cells [13]. The accelerated extraction of flavonoids by increasing microwave power is related to the direct effects of microwave energy on biomolecules by ionic conduction and dipole rotation which result in power dissipated inside the solvent and plant material and then generate molecular movement and heating [14]. More electromagnetic energy was transferred to the extraction system quickly and improved the extraction efficiency when the microwave power increased from 200 to 1000 W.

3.3. Effect of extraction cycles

The effect of repeated and successive extractions of the residue, i.e. extraction cycle, was investigated in this experiment (see Fig. 3). An amount of 5.0 g drug powder was extracted with 50 ml aqueous ethanol at 90 °C for 10 min under solvent to material ratio 10 and 20 ml/g. The residue was taken back and re-extracted three times using fresh solvent each time under the above-mentioned conditions. The yield of flavonoids in three cycles was 0.896 mg/g (at 10 ml/g) and 0.974 mg/g (at 20 ml/g), respectively. The yields of two cycles accounted for 85.9% (at 10 ml/g) and 92.1% (at 20 ml/g) of the yield of three cycles, respectively. There were almost no more flavonoids extracted with the third extraction, especially with the solvent to material ratio 20 ml/g. To save time and energy, two-cycle extraction is enough to release most of the flavonoids.

3.4. Effect of aqueous ethanol concentration

Drug powder weighing 5.0 g was extracted with 50 ml aqueous ethanol at 90 °C for two 10 min cycles. The concentration of aqueous ethanol varied from 60% to 100%. Fig. 4 shows that the yield of flavonoids was greatly influenced by the aqueous ethanol concentration. The yield of flavonoids increased with the increase of ethanol concentration significantly when the ethanol volume percentage in the solvent was lower than 90% (v/v). When extracted with anhydrous alcohol, i.e. 100% (v/v) ethanol, the extraction yield decreased sharply. From these results, it is clear that the addition of some amount of water improved the extraction efficiency. One possible reason for the increased efficiency with a presence of some water might be the increase in swelling of plant material by water, which increased the contact surface area between the plant matrix and the solvent [16–19]. Therefore, 90% (v/v) aqueous ethanol concentration was used in the following experiments.

3.5. Effect of extraction temperature

An amount of 5.0 g drug powder was extracted with 50 ml aqueous ethanol for two 10 min cycles at different temperatures. Fig. 5 shows that the yield of flavonoids increased remarkably with the
increase of temperature from 90 to 110 °C. When above 110 °C, the yield increased slowly and the extract was scorched obviously.

In a closed microwave vessel used in this study, the temperature of the solvent could be increased above the boiling point temperature. As a result, the solubility of flavonoids could greatly be enhanced. The increasing temperature may also cause opening cell matrix, and as a result, flavonoids availability for extraction increases. Moreover, at high temperature, solvent viscosity decreases and the diffusivity increases, thus, the efficiency of extraction increases [20,21]. As high temperature over 110 °C affect the stability of flavonoids as shown in Fig. 1 and to avoid scorch under high temperature 110 °C was chosen for the optimized temperature for the extraction.

3.6. **Effect of irradiation time**

Drug powder weighing 5.0 g was extracted with 50 ml 90% aqueous ethanol at 90 °C for two cycles. Duration of microwave radiation of 5, 10, 15, 20, 25, and 30 min were studied. It is seen in Fig. 6 that the yield of flavonoids at the beginning increased with the increase of duration of microwave radiation and reaches its maximum 1.033 mg/g at 25 min, then fell down slightly. Therefore, the best microwave radiation time is 25 min. Overexposure in the microwave may cause the loss of flavonoids. This was also observed in the extraction of aromatic amines from leather, where the recovery of some amines decreased with irradiation time increasing [22] and similar results was obtained in the extraction of triterpenoid saponins from *Ganoderma atrum* [23]. Therefore, 25 min was chosen as the optimal time for MAE.

3.7. **Effect of solvent to material ratio**

Generally in conventional extraction techniques a higher volume of solvent will increase the recovery, but in MAE a higher solvent volume may give lower recoveries [17,22,23]. To investigate the influence of solvent to material ratio on yield of flavonoids, several grams were extracted for 10 min with 50 ml of solvent at different ratios of solvent to material (10, 15, 20, 25, 30, 35, 40 ml/g, respectively). It is seen in Fig. 7 that the yield of flavonoids increased with the increase of solvent to material ratio and reached its maximum 1.019 mg/g at 30 ml/g. It decreased as the ratio was above 30 ml/g. This was probably due to the larger volume of 90% ethanol causing excessive swelling of the material by water and absorbing the effective constituent [17]. As there was no significant difference between the yield at 25 and that at 30 ml/g, the value of 25 was considered as the optimal ratio of solvent to material for the MAE process.

3.8. **Comparison of MAE with Soxhlet extraction, HRE and ultrasound-assisted extraction**

Soxhlet is recommended for the quality control of *Radix Astragali* in the state pharmacopoeia [24] and ultrasound-assisted extraction was recently used as an alternative to Soxhlet for the flavonoid con-

![Fig. 5. Effect of extraction temperature on flavonoids yield. Extraction conditions: 1000 W, 90% ethanol, solvent ratio 10 ml/g, 10 min, two cycles.](image)

![Fig. 6. Effect of duration of irradiation on flavonoids yield. Extraction conditions: 1000 W, 90% ethanol, solvent ratio 10 ml/g, 90 °C, two cycles.](image)

![Fig. 7. Effect of solvent ratio on flavonoids yield. Extraction conditions: 1000 W, 90% ethanol, 90 °C, 10 min, two cycles.](image)

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of MAE with other extraction methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Extraction methods</td>
</tr>
<tr>
<td>1</td>
<td>Soxhlet</td>
</tr>
<tr>
<td>2</td>
<td>UAE</td>
</tr>
<tr>
<td>3</td>
<td>HRE</td>
</tr>
<tr>
<td>4</td>
<td>MAE</td>
</tr>
</tbody>
</table>
tent analysis in\textit{Radix Astragali} \cite{8}. Heat reflux is the most common method for the extraction of bioactive components from natural products. It can be seen in Table 2 that the flavonoids yield of Soxhlet method is maximum among the four compared methods and MAE is the second highest yield method. Though the flavonoids yield was slightly lower than that of Soxhlet, MAE took only one by fourth time of Soxhlet and the extraction solvent (90\% ethanol) was much safer than methanol used in Soxhlet extraction. The flavonoid yield of MAE is much higher than that of HRE for two 2 h and that of ultrasound-assisted extraction. Therefore, MAE can save a lot of time as compared to Soxhlet and heat reflux method and bring higher yield of flavonoids than HRE and ultrasound-assisted extraction. It is worth noting that MAE is a good alternative to ultrasound-assisted extraction and HRE in the practical production of \textit{Radix} falvonoids.

4. Conclusion

An efficient MAE process has been developed for fast extraction of flavonoids from \textit{Radix Astragali}. The yield of flavonoids under the optimal extraction condition (microwave power: 1000 W, ethanol concentration: 90\%, extraction temperature: 110 °C, irradiation time 25 min, and solvent to material ratio 25 ml/g) was 1.190 ± 0.042 mg/g. Compared to traditional methods, the determined MAE process reduced extraction time, and obtained high percentage extraction of flavonoids without degradation. This showed great potential for industrial application in the near future.

Acknowledgment

We greatly acknowledge the financially support by the Agro-processing Project 2001B501A30 in the Tenth Five-Year Plan of PR China.

References

\[1\] T. Shizuo, S. Yoshihito, Inhibitory effects of \textit{Astragali Radix}, a crude drug in oriental medicines, on lipid peroxidation and protein oxidative modification by copper, J. Ethnopharmacol. 68 (1999) 331–333.

\[2\] T. Shizuo, Antioxidant effects of \textit{Ogikeishigomotsuto}, \textit{Astragali Radix} (Ogi) and isoflavones in \textit{Astragali Radix} on oxidative stress in vitro, J. Tradit. Med. 22 (2005) 162–166.


\[10\] A. Brachet, P. Christen, L. Veuthey, Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves, Phytochem. 11 (2002) 162.


