The Extraction and Determination of Phenolic Compounds in Oil-tea Camellia Seed Oil by Multiwalled Carbon Nanotubes

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Abstract. A reliable, sensitive and effective method based on multiwalled carbon nanotubes (MWs) has been developed to identify and quantify phenolic compounds from oil-tea camellia seed oil (OTCSO). The total phenolic capacity (TPC) was detected to choose and optimize the type of MWs and the influence parameters including the quantity of the adsorbent, the type and volume of desorption solution, the time of extraction and desorption. Use compensation coefficient the recovery of the optimized method presented in OTCSO were between 86.7 % and 111.2 % for phenolic compounds and a satisfactory precision with relative standard deviations (RSD %) lower than 8.31 % for repeatability and reproducibility. In the range 1.02-50.47 µg/g, the method showed good linearity. After method validation, the method was successfully applied to the analysis of OTCSO samples. To our knowledge, this is the first time that phenolic compounds have been extracted from OTCSO using MWs. Our results suggest that MWs offers an efficient, safe, sustainable, and cost effective alternative to conventional method for extraction of phenolic compounds from OTCSO.

Introduction

Carbon nanotubes (CNTs) are carbon allotropes with an interesting nanostructure based on honey comb type graphene sheets (consisting of C-C sp2 bond, one of the strongest bonds of nature) rolled-up in cylindrical nanotubes, are considered the building blocks of new advanced biomaterials [1]. Since their discovery, CNTs have attracted the attention of the scientific world due to their unusual properties. Their high surface area, small diameter, various bulks and individual morphology plus their defected and easily functionalized surface are beneficial for CNTs to become a potential adsorbent for liquid adsorption [2]. By now, CNTs have been used as adsorbents for different types of compounds such as inorganic compounds (Cu (II), Cr (VI), and Zn (II) and organic compounds (methylene blue [3], phenolic compounds, ciprofloxacin [4], natural organic matter [5], nitroaromatic compounds, etc).

Camellia (Camellia oleifera Abel., Theaceae) are widely grown in the south of China, and oil-tea camellia seed oil (OTCSO) is listed among the key healthy edible oils of FAO (Food and Agriculture Organization of the United Nations). Experimental evidences have shown that camellia oil possess a wide array of therapeutic properties including antiseptic, antimicrobial [6], anti-inflammatory [7], antioxidant [8],and so on. The antioxidant stability should be attributed to its unique fatty acid composition, and natural antioxidants, included phenolic compounds. Phenolic compounds which is a broad term used to define substances that possess a benzene ring bearing one or more hydroxyl groups, including functional derivative. Considering the biological importance of these classes of compounds, it would be very interesting to develop methods for their recovery from OTCSO for bioassay tests or medical application. The methods have been reported for the extraction of phenolic
compounds, included conventional liquid-liquid extraction (LLE) [9], solid phase-extraction (SPE) [10] techniques, and several new techniques such as microwave-assisted extraction [11], ultrasound-assisted extraction (UAE) [12], ultrasound-assisted emulsification–microextraction (USAEME) [13], supercritical fluid extraction, and deep eutectic solvent (DESs) [14]. However, from the view point of green chemistry, organic solvent is a volatile, flammable, and toxic liquid. Furthermore, its production mainly relies on non-sustainable natural gas as a feedstock [15].

The aim of the current work was to develop a simple, safe, sensitive and cost-effective method for the analysis of phenolic compounds belonging to the most representative chemical groups present in OTCSOs. The extraction technique with the help of multiwalled carbon nanotubes (MWs) in this manuscript was combines the benefit of LLE and SPE. The optimal extraction conditions were obtained and the quantification of the target analytes were carried out by UV, instrumentation easily available in most analytical laboratories.

Materials and Methods

Chemicals and Samples

Commercial phenolic compound standards such as gallic acid, 3-hydroxytyrosol, protocatechuic acid, chlorogenic acid, homovanillic acid, catechins, (−)-epicatechin, p-coumaric acid, ferulic acid, benzoic acid, coumarin, o-coumaric acid, luteolin, and cinnamic acid were supplied by Sigma-Aldrich Corporation (St. Louis, MO, USA). Stock standard solutions of individual compounds were prepared at 1000 mg L$^{-1}$ in methanol (MeOH) and stored at -20°C. Multicompound working standard solutions were prepared in MeOH by dilution of the stock solutions and stored in amber coloured vials at -20°C. Other chemicals used, included acetone, n-hexane, and water (H$_2$O), are of analytical reagent grade or better.

The OTCSO samples were obtained from high-quality oil-tea camellia seeds collected in November 2015. The oil samples were stored in dark-brown glass bottles without headspace at 10 ± 2°C until analysis. A refined OTCSO supplied by Kangneng Health food co., LTD. were used for matrix effect assessment and recovery studies.

MWs

Table 1. Properties of MWCNTs.

<table>
<thead>
<tr>
<th>MW</th>
<th>Outer diameter</th>
<th>-OH (wt%)</th>
<th>-COOH (wt%)</th>
<th>Special surface area (SSA) ($m^2 g^{-1}$)</th>
<th>Tap density (g cm$^{-3}$)</th>
<th>Purity (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW8</td>
<td>&lt;8</td>
<td>-</td>
<td>-</td>
<td>&gt;350</td>
<td>0.27</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW8-OH</td>
<td>&lt;8</td>
<td>5.58</td>
<td>-</td>
<td>&gt;400</td>
<td>0.27</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW8-COOH</td>
<td>&lt;8</td>
<td>-</td>
<td>3.86</td>
<td>&gt;400</td>
<td>0.27</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW10</td>
<td>10-20</td>
<td>-</td>
<td>-</td>
<td>&gt;180</td>
<td>0.22</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW10-OH</td>
<td>10-20</td>
<td>3.06</td>
<td>-</td>
<td>&gt;170</td>
<td>0.22</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW10-COOH</td>
<td>10-20</td>
<td>-</td>
<td>2.00</td>
<td>&gt;200</td>
<td>0.22</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW20</td>
<td>20-30</td>
<td>-</td>
<td>-</td>
<td>&gt;110</td>
<td>0.28</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW20-OH</td>
<td>20-30</td>
<td>1.76</td>
<td>-</td>
<td>&gt;110</td>
<td>0.28</td>
<td>&gt;95</td>
</tr>
<tr>
<td>MW20-COOH</td>
<td>20-30</td>
<td>-</td>
<td>1.23</td>
<td>&gt;110</td>
<td>0.28</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

MWs with different outer diameters (<8 nm: MW8; 10-20 nm: MW10; 20-30nm: MW20), hydroxylated MWCNTs (MW8-OH, MW10-OH, MW20-OH) and carboxylated MWCNTs (MW8-COOH, MW10-COOH, MW20-COOH) were purchased from Chengdu Organic Chemistry Co. (Sichuan, China), and used as received. These MWCNTs were synthesized from...
ethylene/nitrogen (C2H4/N2 = 3/2) mixture by chemical vapor deposition at 823K using Fe/Al2O3 catalyst. Preliminary experiments indicated that impurities of phenolic compounds in all MWs were below the detection limits. The properties of MWs are listed in Table 1. Before extraction, a previously developed procedure was adapted to the MWs, which is based on the addition of a solution of 1M HCl for 2h, sonication, dilution with distilled water until neutral pH, filtration and drying under vacuum.

**The Total Phenolic Capacity (TPC)**

The concentration of TPC in different extracts was measured using UV spectrophotometer, based on a colorimetric oxidation/reduction reaction using Folin-Ciocalteu reagent. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated, and the results were expressed as mg GAE g−1 extract.

**Characterization Methods**

Fourier transform infrared micro-spectroscopic (µ-FTIR) spectra were recorded on NICOLET iN 10 MX (Thermo Scientific). Samples for µ-FTIR analysis were prepared with identical conditions to that used in the sorption experiments. MWs and phenolic compounds-adsorbed MWs were washed with above mentioned background solution and air-dried overnight. Microsamples were pressed on a diamond bracket and µ-FTIR spectra were measured. FTIR spectra of MWs are the average of 120 accumulated scans using a nominal resolution of 16 cm−1 due to very weak signals of MWs. Characterisation of the MWs was carried out using X-ray photoelectron spectroscopy (XPS) with a Thermofisher K-Alpha XPS system and a monochromated Al K-alpha source (E= 1486.6 eV). High resolution XPS spectra were processed and analysed using CasaXPS (version 2.3.16, Casa Software Ltd.). The Zeta potential was measured by electrophoresis using a Zetameter (Zetameter System 3.0+ model, made by Zetameter Company, Inc., USA) for a suspension of 1g L−1 powder in 10 mg L−1 NaCl.

**Extraction and Desorption of Phenolic Compounds from OTCSO**

The phenolic extract of OTCSO was obtained by the following procedures. Briefly, 40 mL of glass tube sealed with Teflon-lined screw caps was used as batch reactors. Fig 1. Shows the technological process of the extraction and desorption with MWs. Experimental recoveries of batch equilibrium experiments were evaluated without MWs. The average recovery of each adsorbate was 80.54% (rsd=6.12%, n=5). Therefore, the adsorbed phenolic compounds by MW were calculated by mass differences between their initial and final concentrations divide 80.54%. Since compounds with more than one hydroxyl group are more difficult to desorb, great efforts were made to optimize the desorption conditions [1]. The MWs was centrifugal separated and vacuum dried, followed by immersed in acetone by shaking in a vortex for a few seconds, and then the tube was placed

![Figure 1. Sampling of the Phenolic Compounds from OTCSO.](image1.png)

![Figure 2. µ-FTIR Spectras of MW8 (a), MW10 (b), MW20 (c), without and with Phenolic Compounds Adsorbed.](image2.png)
immediately into an ultrasonic water for 30 min. The average recovery of desorption was 61.13% (rsd=5.78%, n=5).

**Result and Discussion**

**µ-FTIR Analysis**

To reveal the adsorption sites and verify the existence of hydrogen bonding of phenolic compounds, a µ-FTIR study was performed. The µ-FTIR spectra of MW, MW-OH, and MW-COOH in the frequency range of 400–4000cm$^{-1}$ are shown in Fig. 2.

As can be seen in Fig. 2 the peaks at 1000-1250m$^{-1}$ can be assigned to C-O stretching vibration, the peaks at 1400 cm$^{-1}$ for –COO- to asymmetric stretching vibration of carboxylic bond, the peaks at 1640cm$^{-1}$ to C=O stretch of keto or carboxyl groups. Other absorption bands are located at 1070cm$^{-1}$ due to C-H in-plane bending vibration of benzene ring. After phenolic compounds was adsorbed to MW8, MW10 and MW20, the C–O stretching vibration peaks at 1165cm$^{-1}$ (Fig. 4a, black line) and 1181cm$^{-1}$ (Fig. 4b, black line) [17]were shifted to 1070cm$^{-1}$ (Fig. 4a, red line) and 1184cm$^{-1}$ (Fig. 4b, red line), respectively, which can be assigned to C-H in-plane bending vibration of benzene ring at 1189cm$^{-1}$ of phenolic compounds and the interaction between the C-O and the benzene ring. These new appeared peaks and the band shifts on MWs suggested that phenolic compounds was partly adsorbed on surface of MWs by hydrogen bonding through the C=O, or –COO- groups.

**Zeta Potential of MWs**

Zeta potential measurements showed that most of MWs became increasingly negatively charged with the adsorption of phenolic compounds compared to few MW like MW10-COOH and MW20-OH became increasingly (Fig. 3).

**XPS Analysis**

The wide scan XPS spectrum (Fig.4 and Fig.5) shows the presence of only carbon and oxygen in the MWs, at different at.% respectively. The high resolution XPS scan of the carbon 1s region can be fit with several components representing C=C (284.7 eV); C-C (286.2 eV, FWHM 2 eV); C-O-C/C-OH (287.1 eV, FWHM 2.2 eV); C=O (287.5 eV, FWHM 2 eV) and O=C-OH (289.3 eV) bonding types. These values are all shifted to ca. 1 eV higher binding energy that might be expected from comparison with literature values. This can be attributed to some charging of the insulating sample during the XPS experiment.
Optimization of the MW Extraction and Desorption Procedure

The adsorption procedure is depending on various factors. Preliminary experiments with spiked commercial OTCSOs were performed in order to evaluate and establish the optimal conditions for the following adsorption affecting variables: diluent solvent, extractant solvent, pH of the extractant solvent, and number of extraction steps. Adsorption isotherms of phenolic compounds on the MWs are depicted in Fig. 6.

The results suggested that the adsorption of phenolic compounds increased with increasing surface areas and pore volumes of MW. MW8 had the largest adsorption amount for all phenolic compounds because MW8 has the largest surface area and pore volume. But the adsorption capacity and efficiency of all MWs performance well (Fig.6), so the choose of the MW was relied on other conditions such as desorptive efficiency.

The desorption procedure is depending on various factors, such as type and dosage of stripping liquid, time, temperature, number of times, and most of all characteristic of MWs. The desorption efficiency of the six MWs were shown in Fig. 7. So, MW20-COOH which all performance was chosen for extraction of phenolic compounds OTCSOs in the following experiments.

Recovery Study and Real Sample Analysis

The proposed method was applied to determine the phenolic contents in three OTCSOs obtained from the camellia varieties described previously in Section 2.5. Table 2 reports the contents of the phenolic compounds determined.

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Initial (µg/g ± S.D.)</th>
<th>Added (µg/g ± S.D.)</th>
<th>Extracted (µg/g ± S.D.)</th>
<th>Recovery (% , n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTCSO 1</td>
<td>30.25 ± 8.31</td>
<td>19.08</td>
<td>47.61 ± 7.32</td>
<td>96.5</td>
</tr>
<tr>
<td>OTCSO 2</td>
<td>10.35 ± 3.15</td>
<td>12.72</td>
<td>20.01 ± 2.57</td>
<td>86.7</td>
</tr>
<tr>
<td>OTCSO 3</td>
<td>8.37 ± 2.31</td>
<td>6.36</td>
<td>16.38 ± 2.68</td>
<td>111.2</td>
</tr>
</tbody>
</table>

Figure 5. High Resolution C 1s XPS Spectrum with Constituent Peaks Adsorbed.

Figure 6. Adsorption of Phenolic Compounds on MWs.

Figure 7. Desorption of Phenolic Compounds on MWs.
As can be seen from Table 2, the reproducibility of the method is satisfactory with relative standard deviations being less than 9% in all cases. MW therefore considered satisfactory and were carried out in further analyses. No great differences were observed for the first compound in the three OTCSOs. Use compensation coefficient the recovery of the optimized method presented in OTCSO were between 86.7% and 111.2% for phenolic compounds and a satisfactory precision with relative standard deviations (RSD %) lower than 8.31% for repeatability and reproducibility. The method showed good linearity and limits of detection and quantification were in the range 1.02-50.47 µg/g, respectively.

Conclusions

We have demonstrated the successful capacity of MW for the extraction of phenolic compounds from OTCSO. Under the extraction conditions used in this paper, almost all the types of MW studied had an enhanced performance for the extraction of the phenolic compounds in OTCSO. Our results propose MWs as both a greener and more efficient alternative to methanol for the large-scale extraction of bioactive compounds from OTCSO. The adsorption of phenolic compounds was closely correlated with surface areas and micropore volumes of MW. Of them, MW10 had the highest adsorption capacity, but MW20-COOH although the adsorbed phenolic compounds were lower, have a better desorption effect. Use compensation coefficient the recovery of the optimized method presented in OTCSO were between 86.7% and 111.2% for phenolic compounds and a satisfactory precision with relative standard deviations (RSD %) lower than 8.31% for repeatability and reproducibility.

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References


