Preparation, characterization and antioxidant activities of acetylated polysaccharides from Cyclocarya paliurus leaves

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ABSTRACT

In this study, polysaccharides extracted from Cyclocarya paliurus leaves were modified to obtain its three acetylated derivatives, Ac-CP1, Ac-CP2, and Ac-CP3. The physicochemical characteristics and antioxidant activities of acetylated derivatives were investigated. The results of chemical and FT-IR spectrum analysis showed differences between acetylated derivatives and native C. paliurus polysaccharide, which revealed that the acetylation were successful. Relative to unmodified polysaccharide, the protein contents of acetylated derivatives decreased, while carbohydrate values increased. The molecular weight (Mw) of acetylated derivatives were approximately 1.05–1.09 × 10^6 Da and were mainly composed of Ara, Gal, Glc, Man, GalA. Ac-CP1, with relatively low degree of substitution (0.13 ± 0.01) exhibited excellent antioxidant activity in DPPH radical assay (95.21 ± 0.89%), and also had strong chelating activity on β-carotene–linoleic acid assay (34.64 ± 2.07%) at 0.5 mg/mL. In addition, scanning electron microscope (SEM) observations suggested that acetylation could change the morphology and structure of polysaccharides from C. paliurus leaves.

1. Introduction

Polysaccharides as natural polymers, widely existed in plants, animals and microorganisms, have a variety of biological properties, such as immunomodulatory, anti-inflammatory and anti-tumour activities (Simpson & Morris, 2014; Yi et al., 2012; Wang, Zha, Pan, & Luo, 2013; Zhang, Cui, Cheung, & Wang, 2007). Nowadays more and more attention was cast on chemical modification of polysaccharides in order to improve their intrinsic biological functions and to obtain new pharmacological agents with possible medical uses. Acetylation is known to be an important modification method to modify the physicochemical properties, and bioactivities of natural polysaccharides. In the acetylation, parts of the hydroxyl groups of the polysaccharides units have been converted by esterification to acetyl groups (Singh, Nath, & Guha, 2011). Acetylated starch has many unique properties, such as an increase in solubility, swelling power, clarity, reduced the gelatinization temperature, and also decreased the tendency toward retrogradation (Schlemmer, Angelica, & Sales, 2010). Acetylation of mannans or other polysaccharides can be used to control their solubility, water absorbency, hydrophobicity, and physical properties (Penroj, Mitchell, Hill, & Ganjanagunchorn, 2005; Williams et al., 2000; Xu et al., 2010). Song et al. (2013) have also found that acetylated pumpkin polysaccharide showed relevant higher antioxidant activity that of unmodified polysaccharide both in vitro and in a H2O2-induced cell system.

Cyclocarya paliurus (Batal.) Iljinskaja (C. paliurus), known as “sweet tea tree”, belongs to the Juglandaceae family, which grows in mountainous regions. The leaves of C. paliurus have been used to make tea in China because of its unique taste. The leaves possess several health benefits, such as antihypertensive activity, enhancement of mental efficiency and antioxidant activity (Kurihara et al., 2003; Xie et al., 2012). It is also used to inhibit inflammation and prevent hypolipidaemic and diabetes (Xie et al., 2010). In 2013, C. paliurus was approved as new food raw material by National Health and Family Planning Commission of China (Xie et al., 2015). Polysaccharide was considered as one of the effective bioactive components in the leaves of C. paliurus. Great progresses had been made on the studies of composition, physicochemical properties, and bioactivities of C. paliurus polysaccharide in recent years (Huang, Nie, Xie, Han, & Xie, 2008; Xie et al., 2010, 2012, 2013a). In our previous work (Xie, Liu et al., 2013), one immunoregulatory polysaccharide (CPP), with a molecular weight of 9.0 × 10^5 Da, was obtained from the leaves of C. paliurus. Structure feature of...
the purified polysaccharide was investigated by a combination of chemical and instrumental analysis. Preliminary tests showed that CPP exhibited strong growth inhibitory activities on human gastric cancer cells (Xie, Liu et al., 2013), and had potent stimulating effects on murine lymphocyte proliferation (Huang et al., 2009).

The bioactivities of polysaccharides mainly depends on their physicochemical properties, such as degree of substitution, molecular weight, monosaccharide composition, polysaccharide content, type of sugar and functional groups (Melo, Feitosa, Freitas, & de Paula, 2002; Zhang et al., 2007). The structure and antioxidant activity of C. paliurus polysaccharide had investigated in our previous work (Xie et al., 2010, 2012). However, to our knowledge, research on acetylation of C. paliurus polysaccharide has not been reported so far, and the antioxidant activity of the acetylated polysaccharide derivatives still unknown. Therefore, the aim of the present paper was to investigate the acetylation of polysaccharide isolated from the leaves of C. paliurus and to compare their antioxidant activities with unmodified polysaccharide. The new acetylated derivatives were characterized by different methods such as high-performance gel permeation chromatography (HPGPC), high-performance anion exchange chromatography (HPAEC), UV–vis and Fourier transform infrared (FT-IR) spectroscopies. Then, their antioxidant activities were investigated by scavenging effect of DPPH radicals and β-carotene–linoleic acid assay.

2. Materials and methods

2.1. Plant materials

The leaves of C. paliurus were collected from Jiangxi Province of China. All the samples were authenticated by Prof. Zhihong Fu in Jiangxi University of Traditional Chinese Medicine, China. Voucher specimen was deposited at the State Key Laboratory of Food Science and Technology, Nanchang University, China. The materials were air-dried and ground into a fine powder in a mill before extraction.

2.2. Chemicals and reference compounds

Glucose (Glc), xylose (Xyl), arabinose (Ara), rhamnose (Rha), galactose (Gal), ribose (Rib), mannose (Man), fucose (Fuc), galacturonic acid (GaA) and glucuronic acid (GlcA) were purchased from Sigma Chemical Co. (St. Louis, USA). The water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Other reagents used in the study were of analytic grade.

2.3. Preparation of C. paliurus polysaccharides

The polysaccharide was extracted from the leaves of C. paliurus as previously described (Xie, Shen, Nie, Li, & Xie, 2011). Briefly, the dried C. paliurus leaves (1000 g) were first extracted with 80% ethanol (v/v) for 24 h at room temperature to remove coloring matter and the extraction was repeated twice. After the mixture was filtered, the residues were dried in air, the dried ethanol extracts were extracted with hot water at 80 °C for twice, 3 h each time, filtered and centrifuged to remove water-insoluble materials. The aqueous extract was concentrated to 20% of the original volume with a rotary evaporator at 55 °C under vacuum. The proteins in the extract were removed using the Sevag reagent. The concentrated solution was precipitated with 80% (v/v) ethanol. The supernatant was collected and then kept at 4 °C overnight in refrigeration; finally polysaccharide pellets were obtained by centrifugation at 8400 × g for 15 min and repeatedly washed sequentially with anhydrous ethanol, acetone and diethyl ether. The refined polysaccharide pellets were completely dissolved in an appropriate volume of distilled water and intensively dialysed for 2 days against distilled water (MWCO 14,000 Da). Finally, the C. paliurus polysaccharide (CP) was obtained by lyophilization.

2.4. Preparation of acetylated polysaccharides

The acetylated polysaccharides were prepared according to the method reported by Chen et al. (2014) with some modifications. Briefly, CP (500 mg) was dissolved in 15 ml distilled water. The mixture was stirred with a magnet stirrer until homogeneous solution was obtained. The pH was adjusted to 9.0 with 10 M NaOH. The mixture was stirred at room temperature for 4 h. During this period, the required amount of acetic anhydride (1, 4 and 6 ml) was added drop by drop, respectively, at 50 min intervals. While simultaneously, 10 M NaOH was added to the mixture with continuous stirring to maintain the pH at 8.0–8.5. After reaction, the pH of the solution was neutralized to 7 with 10 M HCl to terminate the reaction. The polysaccharides solution was dialyzed against tap water for 36 h and against ultra pure water for 12 h (Mw cut-off 14 kDa). The aqueous solution was precipitated with 95% (v/v) ethanol at 4 °C for 12 h, and then the resulting precipitate was dissolved in distilled water and freeze dried.

2.5. Degree of substitution

The acetyl group and the degree of substitution (DS) of Ac-CPS were determined as described by Sánchez-Rivera et al. (2010). A 20 mg sample of acetylated polysaccharide was weighed, transferred to a 250 ml flask and dispersed in 10 ml 0.01 M NaOH. The loosely stepper flask was agitated, warmed to 50 °C for 2 h. The excess alkali was back-titrated with 0.01 M HCl to disappearance of pink color using phenolphthalein as an indicator. The original unmodified C. paliurus polysaccharide (CP), was used as a blank. Initially, the acetyl group (%) was calculated as:

$$\text{Acetyl group(%) = } \frac{[\text{V}_1 - \text{V}_2] \times M \times 0.043 \times 100}{W}$$

(1)

where V₁ is the volume of 0.01 M HCl used to titrate blank (ml), V₂ is the volume of 0.01 M HCl used to titrate sample (ml), M is the molarity of HCl, and W is the mass of sample (g).

The DS is defined as the average number of sites per glucose unit that possess a substituent group (Whistler & Dännel, 1995). The DS was calculated as:

$$\text{DS = } \frac{162 \times \text{Acetyl group(%)}}{4300-42 \times \text{Acetyl group(%)}}$$

(2)

2.6. Characterization

Infrared spectra (IR) analysis of the native and acetylated derivatives were recorded with a Nicolet 5700 FT-IR (Thermo Fisher Scientific Inc., MA, USA) spectrometer in the range 4000–500 cm⁻¹ using the KBr-disk method. UV–vis spectra were performed on an UV–vis spectrophotometer (TU-1900, Pgenenal, Beijing, China) at 25 °C in the range of 190–400 nm using a quartz cell with 1 cm path length. Analysis of the morphology of the native and acetylated sample derivatives were carried out using scanning electron microscope (JEOL, Japan), from Institute of Chemical Technology.

Total carbohydrate content was determined by phenol-sulfuric acid method. Protein content was measured with coomassie brilliant blue method (Lowry, Rosebrough, Lewsfarr, & Randall, 1951).
Uronic acid content was determined by the method (Blumenkrantz & Asboe Hansen, 1973). Data within a column without the same superscripts (a–d) differ significantly (\( P < 0.05 \)).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Ac-CP1</th>
<th>Ac-CP2</th>
<th>Ac-CP3</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yield (%)</strong></td>
<td>68.78±3.11a</td>
<td>73.12±2.72a</td>
<td>86.75±3.08b</td>
<td>86.75±3.08b</td>
</tr>
<tr>
<td><strong>DS</strong></td>
<td>0.13±0.01a</td>
<td>0.28±0.03b</td>
<td>0.57±0.07c</td>
<td>0.57±0.07c</td>
</tr>
<tr>
<td><strong>Chemical composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total carbohydrate (%)</strong></td>
<td>64.89±0.76a</td>
<td>66.17±1.03a</td>
<td>66.91±0.82b</td>
<td>60.62±0.88a</td>
</tr>
<tr>
<td><strong>Protein (%)</strong></td>
<td>7.25±0.12a</td>
<td>6.93±0.08a</td>
<td>7.09±0.16a</td>
<td>7.57±0.26a</td>
</tr>
<tr>
<td><strong>Uronic acid (%)</strong></td>
<td>15.78±1.09a</td>
<td>25.99±0.57b</td>
<td>27.43±0.76b</td>
<td>16.14±0.44a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monosaccharide composition (molar ratio of monosaccharide, mol/mol)</th>
<th>Ac-CP1</th>
<th>Ac-CP2</th>
<th>Ac-CP3</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ara</strong></td>
<td>1.00a</td>
<td>1.00a</td>
<td>1.00a</td>
<td>1.00a</td>
</tr>
<tr>
<td><strong>Gal</strong></td>
<td>1.67a</td>
<td>1.68a</td>
<td>1.93b</td>
<td>1.59a</td>
</tr>
<tr>
<td><strong>Glc</strong></td>
<td>1.07a</td>
<td>0.93a</td>
<td>0.63b</td>
<td>1.81a</td>
</tr>
<tr>
<td><strong>Rha</strong></td>
<td>0.15a</td>
<td>0.18a</td>
<td>0.14a</td>
<td>0.08b</td>
</tr>
<tr>
<td><strong>Xyl</strong></td>
<td>0.11a</td>
<td>0.14a</td>
<td>0.07b</td>
<td>0.35b</td>
</tr>
<tr>
<td><strong>Man</strong></td>
<td>0.34a</td>
<td>0.39a</td>
<td>0.36b</td>
<td>0.48b</td>
</tr>
<tr>
<td><strong>GalA</strong></td>
<td>1.58a</td>
<td>1.98b</td>
<td>2.08b</td>
<td>0.81b</td>
</tr>
<tr>
<td><strong>GlcA</strong></td>
<td>0.16a</td>
<td>0.19ab</td>
<td>0.22b</td>
<td>0.31b</td>
</tr>
</tbody>
</table>

Data within a column without the same superscripts (a–d) differ significantly (\( P < 0.05 \)).

A Values were expressed as mean ± SD and three replicated independent determinations.

B Analyzed by high-performance anion exchange chromatography coupled with pulsed amperometric detection, after acid hydrolysis.

Uronic acid content was determined by the \( m \)-hydroxydiphenyl method (Blumenkrantz & Asboe Hansen, 1973).

Monosaccharide compositions were analyzed by high-performance anion exchange chromatography (HPAEC) as our previous study (Denman & Morris, 2015; Xie, Shen et al., 2013). HPAEC was performed on Dionex ICS-2500 system, coupled with PAD, and a Carbo PAC™ PA10 column (2.0 × 250 mm). Details of the operating conditions and methods were described in details before (Xie et al., 2013). Rha, Ara, Glc, Xyl, Man, Gal, GlcA and GaA were chosen as standards. The average molecular weight was evaluated by HPGPC on Agilent 1260 HPLC system (Agilent, USA) with refractive index and UV detectors. Ultrahydrogel™ 1000 column was eluted with pure water at a flow rate of 0.6 ml/min. Dextrins with different molecular weights were used as standards for calibration.

#### 2.7. Antioxidant activity

##### 2.7.1. DPPH radical scavenging activity assay

The DPPH radical scavenging capacity of the polysaccharide was analyzed according to the method in our previous study (Xie et al., 2010). The reaction mixture comprised 2 ml of DPPH (0.1 mM in 95% ethanol), 0.5 ml of polysaccharide samples with various concentrations and 1.5 ml water. The mixture was shaken vigorously and kept at room temperature for 30 min in the dark, and the absorbance of the mixture was determined at 517 nm with ascorbic acid as the positive control. The antioxidant activity of polysaccharide was evaluated as follows:

\[
\text{Scavenging activity(%) = } \frac{1 - \frac{A_1 - A_0}{A_2}}{100}
\]

here \( A_0, A_1 \) and \( A_2 \) are the absorbance of blank, sample, and negative control (the mixture of 2 ml of DPPH-ethanol solution plus 2 ml ethanol), respectively.

##### 2.7.2. \( \beta \)-carotene–linoleic acid assay

Approximately 10 mg of \( \beta \)-carotene, 40 mg of linoleic acid and 400 mg of Triton X-100 were mixed in chloroform. After removed the chloroform, 100 ml of oxygenated ultra pure water was added slowly to the semi-solid residue with vigorous agitation to form an emulsion (Suja, Jayalekshmy, & Arumughan, 2005). 1.8 ml of \( \beta \)-carotene–linoleic acid emulsion was mixed with 0.2 ml of samples in test tubes, and then subjected to thermal oxidation at 50 °C for 2h. After this incubation period, absorbance of the emulsion was measured at 470 nm. The antioxidant activity of polysaccharide was expressed in% basis of preventing bleaching of \( \beta \)-carotene using the following formula:

\[
\text{Inhibitory activity(%) = } \left(1 - \frac{A_1}{A_2}\right) \times 100\%
\]

where \( A_1 \) and \( A_2 \) are the degradation rate of the control and sample (i.e., \( \ln (a/b)/120 \), where \( a \) is the initial absorbance at time 0, and \( b \) is the absorbance at time 120 min).

##### 2.8. Statistical analysis

The values were expressed as the means ± standard deviation (SD) of three replicates. The data were subjected to an analysis of variance (ANOVA), and the significances of the differences between samples were determined using Duncan’s multiple-range tests for means with 95% confidence limit (\( P < 0.05 \)).

### 3. Results and discussion

#### 3.1. Preparation of Ac-CPs

The crude polysaccharide (CP) was isolated from \( C. paliurus \) leaves by hot water extraction, ethanol precipitation, deproteinization and lyophilization. Physicochemical analysis showed that CP was an acid hetero-polysaccharide, and its carbohydrate content and uronic acid content were 78.6% (w/w) and 26.2% (w/w), respectively. The chemical composition of \( C. paliurus \) polysaccharide and the chromatographic examination of its hydrolysates in our previous work showed the presence of major amounts of Glc, Ara, Man, Gal, uronic acids and minor amount of Rha and Xyl (Xie, Shen et al., 2013).

The procedure of acetylation reaction included two steps. Firstly, sodium hydroxide reacted with the hydroxyl groups of the \( C. paliurus \) polysaccharide to produce alkoxides groups. Secondly, the acetyl groups formed between the \( C. paliurus \) polysaccharide alkoxides and acetic anhydride through reaction. In the present work, three acetylated polysaccharides of \( C. paliurus \) polysaccharide prepared by adding a different amounts from 1 to 6 ml, which were named as Ac-CP1, Ac-CP2, and Ac-CP3, respectively. The DS values of acetylated polysaccharides increased with the increasing acetic...
anhydride amounts for 0.13 ± 0.01 (Ac-CP1), 0.28 ± 0.03 (Ac-CP2) and 0.57 ± 0.07 (Ac-CP3) (Table 1), for 1, 4, and 6 ml acetic anhydride, respectively. Therefore, the acetylated polysaccharides with various DS were successfully prepared.

The yields of acetylated derivatives from C. paliurus polysaccharide obviously increased with increasing ratio of acetic anhydride amounts for 86.75 ± 3.08% (Ac-CP3), 73.12 ± 2.72% (Ac-CP2) and 68.78 ± 3.11% (Ac-CP1) (Table 1). These results are in agreement with the report of acetylated polysaccharides isolated from pumpkin (Song et al., 2013).

3.2. Chemical analysis

The chemical analysis of all the samples was given in Table 1. In general, the total sugars and uronic acid contents of the acetylated polysaccharides increased, whereas protein contents were relatively decreased after modification. The acetyl contents of the derivatives were higher than the native polysaccharide, which indicated the acetylation was successful (Xu et al., 2010).

The chemical composition of Ac-CP1, Ac-CP2 and Ac-CP3 revealed that the total carbohydrate content was 64.89 ± 0.76%, 66.17 ± 1.03% and 66.91 ± 0.82%, respectively. In addition, small amounts of protein were found in Ac-CP1, Ac-CP2 and Ac-CP3, with 7.25 ± 0.12%, 6.93 ± 0.08% and 7.09 ± 0.16%, respectively (Table 1). Ac-CP1 had the least uronic acid content of 15.78 ± 1.09%, and Ac-CP2 (25.99 ± 0.57%) had roughly equal content with Ac-CP3 (27.43 ± 0.76%). Specifically, the content of uronic acid in Ac-CP1 was less than that in CP (16.14 ± 0.44%).

The homogeneity and molecular weight of each purified fraction were analyzed by high-performance gel permeation chromatography (HPGPC). As shown in Fig. 1, the distributions or the range of elution time of the acetylated polysaccharides were comparatively centralized, and the HPGPC profiles of the three acetylated polysaccharides were comparatively single and symmetrical peak. In addition, the average molecular weights of Ac-CP1, Ac-CP2 and Ac-CP3 were estimated to be 1050, 1080 and 1090 kDa, respectively. The results showed that the acetylated derivatives had a similar average molecular weights to that of natural polysaccharide, indicating that the derivatives were modified successfully without degradation in this study.

The UV spectra of the three acetylated derivatives showed significant absorptions at 260–280 nm (Fig. 2), which was consistent with the analytical results of the protein content in all the polysaccharides (Table 1). There were no apparent differences existed among the native C. paliurus polysaccharide and its acetylated derivatives.

The monosaccharide compositions of Ac-CP1, Ac-CP2 and Ac-CP3 were analyzed by HPAEC and the results were shown in Table 1. According to the monosaccharide composition analysis, all the acetylated derivatives were heteropolysaccharides, and Ara, Gal, Glc were the main sugar unit. For neutral sugar, Ac-CP1, Ac-CP2 and Ac-CP3 were mainly composed of Ara, Gal, Glc, Rha and Man in a molar ratios of 1.00:1.67:1.07:0.15:0.34, 1.00:1.68:0.93:0.18:0.39 and 1.00:1.91:0.63:0.14:0.36, respectively. However, all the acetylated derivatives showed lower ratio of Xyl. The monosaccharide composition found by HPAEC was comparable with the literature values of CP (Xie, Shen et al., 2013). Furthermore, the uronic acids...
in the three acetylated derivatives were identified as GalA and GlcA. The molar ratio of GalA and GlcA in Ac-CP1, Ac-CP2 and Ac-CP3 were 1.58:0.16, 1.96:0.19 and 2.08:0.22, respectively. Notably, Ac-CP3 contained much higher content of uronic acid, which was quite different from Ac-CP1 and Ac-CP2. The results were strongly in accordance with the uronic acid contents determined by m-hydroxydiphenyl method. The result obtained from experiment showed that the acetylation modification affects the composition and physico-chemical characteristics of C. paliurus polysaccharide.

3.3. FT-IR analysis

The FT-IR spectra of native polysaccharide (CP) and acetylated polysaccharides are presented in Fig. 3. For all polysaccharides, the broad intense peak at around 3422 cm⁻¹ and narrow weak peak at around 2930 cm⁻¹ in both spectra were attributed to the stretching vibration of −OH and −C−H, respectively. The absorption band at 2912 cm⁻¹ was attributed to −C−H stretching vibrations of the free sugar. The absorption peaks toward about 1616 cm⁻¹ implied the presence of carboxyl groups. And the bands in the region 1350–1450 cm⁻¹ were corresponded to symmetrical deformations of CH₂ and COH groups (Ren, Sun, & Peng, 2008).

Comparison between the FT-IR spectra of acetylated polysaccharides and native polysaccharide, the absorptions at 1240 cm⁻¹ assigned to stretching vibration of the C−O−C in carbonyl groups with the increase of the carbonyl stretching vibration, was increased with an increase of DS value. A band around 1090 cm⁻¹, assigned to C−O and C−O−C stretching, as well as C−OH bending, became sharper after acetylation. These results indicated that acetylation of the polysaccharide proceeded, and the degree of acetylation was enhanced with increase of substitution degree (Song et al., 2013).

3.4. SEM analysis

Previous studies have demonstrated the introduction of acetyl groups could change the structural and physicochemical characteristics of polysaccharide, the aim of this study was to modify C. paliurus polysaccharide and evaluate their physicochemical properties. The changes in surface morphology of polysaccharides were likely to show the result of the modification. SEM is an effective tool to analyze the surface morphology change of native polysaccharide (CP) and acetylated polysaccharides. The structures of native polysaccharide (CP) and acetylated polysaccharides were characterized by SEM, and the results are presented in Fig. 4.

The surfaces of the native C. paliurus polysaccharide (CP) and acetylated polysaccharides showed obvious variations in size and shape, and surfaces of acetylated polysaccharides is not obvious. The SEM of the acetylated polysaccharides derivatives were in the shape of irregular lumps while the surface of native polysaccharide appeared as flake-like morphology under the 1000 fold exaggeration condition. Seen from the image at 5000 fold augmentation, the surface morphology of native C. paliurus polysaccharide exhibited relatively irregular round-like shape, and surface morphology of acetylated polysaccharides was approximate to CP, but became less rough. The results of SEM indicated that the surface form of C. paliurus polysaccharide had a trend to becoming smooth after modification. It was reported that polysaccharides structure could be affected by the strong acid and high temperature in acetylated modification. This variation on morphology might be related to that there was increased intermolecular cross-linking when acetylated modification and the conformational had changed.

3.5. Antioxidant activities of the acetylated C. paliurus polysaccharides

3.5.1. DPPH radical scavenging activity

As a stable free radical, the DPPH free radical has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants. An alcoholic solution of DPPH has a UV–vis absorption maximum at 517 nm. Based on this principle, the DPPH radical scavenging activities of Ac-CP1, Ac-CP2, and Ac-CP3 are shown in Fig. 5a where ascorbic acid was used as a control.

As expected, the scavenging activities of three acetylated C. paliurus polysaccharides derivatives had the scavenging activity on DPPH radicals, which were increased in a dose-dependent way. The scavenging activity of acetylated C. paliurus polysaccharides was significantly higher than that of the native polysaccharide CP. The DPPH radical scavenging activity of the Ac-CP1, Ac-CP2, and Ac-CP3 were similar, 93.55 ± 1.82%, 89.69 ± 0.64%, and 90.27 ± 1.71%, respectively, at 0.25 mg/ml and lower than that of ascorbic acid (95.19 ± 2.33%). In particular, Ac-CP1 at the concentration of 0.5 mg/ml exhibited the highest ability (94.23 ± 1.41%) to quench DPPH radical, which was similar to ascorbic acid (95.21 ± 0.89%). The IC50 values of radical scavenging activity on DPPH were found to be 64.48, 78.29, and 78.04, and 98.37 µg/ml for Ac-CP1, Ac-CP2, and Ac-CP3, respectively. Meanwhile, the IC50 values of Vc in eliminating DPPH radical was about 10 µg/ml. The percent scavenging activity of all fractions from C. paliurus is presented in the following descending order: ascorbic acid > Ac-CP1 > Ac-CP2 > Ac-CP3. The results suggested that all the acetylated C. paliurus polysaccharides derivatives showed stronger inhibition activity than the native polysaccharide CP, which indicated that the acetylated modification increased the DPPH radical scavenging activity of C. paliurus polysaccharide. The Ac-CP3 has higher activity than Ac-CP2, while the DS of Ac-CP2 is higher than Ac-CP3, which indicated that acetylated polysaccharides with proper substitution could be showed better antioxidant activity to inhibit DPPH radicals. These results were consistent with the findings of the antioxidant activities of polysaccharide could be improved after acetylated reported by many research groups (Liu, Luo, Ye, & Zeng, 2012; Song et al., 2013).

3.5.2. β-carotene–linoleic acid assay

Recently, the assay is widely used to evaluate lipid peroxidation inhibitory activity of plant extracts because it is simply and cheap. The test system is based on discoloration of β-carotene in the absence of antioxidants. The presence of an antioxidant can hinder the extent of β-carotene degradation by neutralizing the
linoleate free radical and any other free radicals formed within the system (Moon & Shibamoto, 2009).

The antioxidant capacity of CP and its derivatives as measured by bleaching of β-carotene is shown in Fig. 5b. The antioxidant activity calculated based on the average rate of β-carotene bleaching was relatively high for all the investigated polysaccharide samples. All the polysaccharides significantly inhibited bleaching of β-carotene. In this assay, Ac-CP1 had the best antioxidant activity (74.20 ± 3.62%), and it was similar to that of BHT (79.21 ± 2.19%) at 1.0 mg/ml. At 0.5 mg/ml, Ac-CP1 also showed the greatest antioxidant efficacy (73.88 ± 2.51%), followed by Ac-CP3 (38.67 ± 2.76%) and Ac-CP2 (35.66 ± 3.02%). These results suggested that Ac-CP1 was inhibitory to β-carotene–linoleic acid, and it might be advantageous for preventing injury induced by radicals in pathological conditions. In the same test system, inhibition values of β-carotene–linoleic acid oxidation assay were found to be 29.30% in Ac-PSG–2 of the acetylated polysaccharide from Ganoderma atrum (Chen et al., 2014).

In the metabolism of most organisms, free radicals are continuously produced, which are toxic to human body (Liochev, 2013). A number of polysaccharides have been recently demonstrated to play an important role as free radical scavengers in the prevention of oxidative damage (Liu, Du, Wang, Zha, & Zhang, 2014; Xie et al., 2012). Many studies have demonstrated that the acetylation of natural polysaccharides is an important way of obtaining new antioxidants agents (Liu et al., 2012; Song et al., 2013).

In the present study, acetylated derivatives from the leaves of C. paliurus were found to possess the remarkable antioxidant activity to scavenge DPPH radical and radicals in the β-carotene–linoleic acid system (Fig. 5). The mechanism is that the presence of acetyl groups in the polysaccharide results in a weaker dissociation energy of the O–H bond. Therefore, acetylated polysaccharides possess a greater capacity to donate hydrogen to the superoxide anion because of the weaker dissociation energy of O–H bond. Results of the present study also suggested that the introduction of acetyl groups and their DS could affect the molecular weight, polarity,
Fig. 4. Scanning electron microscopy images of the native C. paliurus polysaccharide (a1, 500 × CP; a2, 20,000 × CP), acetylated C. paliurus polysaccharide Ac-CP1 (b1, 500 × Ac-CP1; b2, 20,000 × Ac-CP1), acetylated C. paliurus polysaccharide Ac-CP2 (c1, 500 × Ac-CP1; c2, 20,000 × Ac-CP2), and acetylated C. paliurus polysaccharide Ac-CP3 (d1, 500 × Ac-CP3; d2, 20,000 × Ac-CP3).
Fig. 5. (a) DPPH radical scavenging activities of the unmodified and acetylated C. paliurus polysaccharides. (b) Superoxide anion radical scavenging activities of the unmodified and acetylated C. paliurus polysaccharides.

4. Conclusions

In the present study, three acetylated derivatives with different DS (0.13–0.57) were successfully prepared from the water soluble polysaccharides extracted from the leaves of C. paliurus. This is the first report on the evaluation of the composition and antioxidant properties of acetylated polysaccharides from C. paliurus. The physicochemical and structural study revealed differences in acetylated derivatives and native C. paliurus polysaccharide, which influenced the physicochemical characteristics and bioactivities. All the acetylated derivatives were found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the DPPH free radical and β-carotene–linoleic acid model system. The carbohydrate, protein and uronic acid content of Ac-CP1 was 64.89 ± 0.76%, 7.25 ± 0.12% and 15.78 ± 1.09%, respectively. In addition, Ac-CP1 with Ds of 0.13 ± 0.01 exhibited 93.55 ± 1.82% DPPH radical scavenging activity. The present study suggested that the introduction of acetyl groups and their DS could affect the molecular weight, chemical, structural and conforma-

tion of native polysaccharides, which might lead to the change of bioactivities. This perspective deserves to be explored by food and pharmaceutical industries to obtain material that can be used either as nutriment, food additives, or antioxidant supplements. Future research should focus on the relationship between the antioxidant mechanism and the structure of the acetylated polysaccharide.

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References


