

## Decolorization of polysaccharides solution from *Cyclocarya paliurus* (Batal.) Iljinskaja using ultrasound/H<sub>2</sub>O<sub>2</sub> process

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### ABSTRACT

An advanced oxidation process, ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation was used for the decolorization of *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharides (CPP). The effects of main operating parameters including initial concentration of CPP solution, dosages of H<sub>2</sub>O<sub>2</sub>, temperature, pH and ultrasonic irradiation on the decolorization efficiency of CPP were investigated and the optimum operational conditions of the process were also evaluated. Furthermore, HPLC, Fourier transformed infrared (FT-IR) and NMR spectra methods were applied to analyze the components and structure changes of CPP. Results showed that ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process represented good decolorizing ability on CPP. The optimum operational conditions were determined as follows: concentrations of the polysaccharide solution, 0.5 mg/ml; H<sub>2</sub>O<sub>2</sub>, 0.623 mM; temperature, 40 °C; pH, 9.0. Under these conditions, the decolorization efficiency was reached to 84.1%. The results of HPLC, FT-IR and NMR analysis indicated that ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process did not result in any significant change in the structure of CPP.

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### 1. Introduction

*Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*), commonly known as 'sweet tea tree', is a medicinal herb, an endemic tree growing on cloudy and foggy highlands in the tropics and subtropics in the south of China (Fang, Wang, Wei, & Zhu, 2006). The leaves of *C. paliurus* have been used in China both as drug formulations in traditional Chinese medicine (TCM) and as an ingredient in health foods or dietary supplements (Xie, Li, Nie, Wang, & Lee, 2006; Xie, Xie, Nie, et al., 2010). Recently, it has been reported that the leaves of *C. paliurus* are used in folk medicine for the treatment of diabetes, hypertension hyperlipidemia, anti-hypertensive (Kurihara, Asami, Shibata, Fukami, & Tanaka, 2003; Li et al., 2000), antioxidant and the enhancement of mental efficiency have also been recorded for the leaves of this plant in our previous study (Liu et al., 2007; Xie & Xie, 2008; Xie, Xie, Shen, et al., 2010). Our earlier study showed that the polysaccharides were one of the main components of the water extracts of *C. paliurus* leaves. The polysaccharides has been found to exhibit a variety of biological activities such as antioxidant activity, antihyperglycemic, antidiabetic, reducing blood sugar and enhancing human nonspecific immunity, etc. (Liu et al., 2007; Xie et al., 2006; Xie, Xie, Nie, et al., 2010; Xie, Xie, Shen, et al., 2010). In our early research, the crude polysaccharides

derived from the leaves of *C. paliurus* are back-brown and contain high pigments, which is a major problem associated with the further isolation, purification, structure identification of polysaccharides derived from higher plants. Therefore, it is necessary to remove the pigments from CPP. However, less attention has been paid to the decolorization of polysaccharides.

Various chemical and physical processes, such as activated carbon adsorption process (Ahmedna, Marshall, & Rao, 2000; Simaratanamongkol & Thiravetyan, 2010), exchange resins adsorption (Achaerandio, Guëll, & López, 2002) and H<sub>2</sub>O<sub>2</sub> oxidation process have been employed for decolorization. However, those treatments possess inherent limitations such as high cost, ineffective, complicated, formation of hazardous by-products, and intensive energy requirements. Therefore, it is necessary to find an effective method of treatment. Among the new oxidation methods called advanced oxidation processes, chemical oxidation using ultrasonic in the presence of H<sub>2</sub>O<sub>2</sub> is a very promising technique. Ultrasound is described as a possible generator of highly active •OH, •HOO, •H radicals (Adewuyi, 2001; Mahmoodi, Arami, Limaee, & Tabrizi, 2005). These radicals are capable of initiating or promoting many fast reduction–oxidation reaction. During the last decade, some investigators have reported the successful applications of the ultrasound/H<sub>2</sub>O<sub>2</sub> process for the decolorization of wastewater (Zhang & Zheng, 2009).

As far as we know, there is little information on the application of ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process for the decolorization of polysaccharides. Therefore, the aim of this work was to study

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the feasibility of applying ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process to remove pigments from polysaccharides, and finally established a reliable technique for the decolorization of polysaccharides isolated from *C. paliurus*. The operating parameters such as initial CPP concentration, H<sub>2</sub>O<sub>2</sub> dosages, temperature and pH value were optimized, and the effects of ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation treatment on the structure of CPP were also evaluated by HPLC, NMR and FT-IR spectra methods.

## 2. Materials and methods

### 2.1. Materials and reagents

The dried leaves of *C. paliurus*, cultivated in Xiushui County, Jiangxi Province, China, were provided by Jiangxi Xiushui Miraculous Tea Industry Co. (Jiangxi, China). All samples were sliced and ground into fine powder in a mill before extraction.

Standard monosaccharide references (arabinose, rhamnose, fucose, xylose, galactose, glucose, ribose and mannose), galacturonic acid and glucuronic acid reference were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The H<sub>2</sub>O<sub>2</sub> solution with 30% (w/w) concentration, NH<sub>4</sub>OH, HCl and ethanol were obtained from Shanghai Chemicals and Reagents Co. (Shanghai, China). All reagents used were of analytical grade. Aqueous solutions were prepared with purified water from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

### 2.2. Preparation of CPP

The dried leaves powder of *C. paliurus* (40 mesh) were first weighed and extracted with 80% ethanol for 24 h to remove the interference components such as monosaccharide, disaccharide, oligosaccharide in the samples at 80 °C. After filtration, the residue were dried at room temperature and placed in an extraction tube, then extracted twice with distilled water at 80 °C for 2 h. The extracts were filtered through glass wool and centrifuged at 8000 × g for 5 min in a high speed centrifuge (3K3D, Sigma, Germany) to separate the supernatant and the residue. The twice extracts were combined and concentrated under reduced pressure at 50 °C and precipitated with four volumes of ethanol, then kept at 4 °C overnight in refrigerator to precipitate polysaccharides. The precipitates formed in the solution were collected and then lyophilized in vacuum freeze dryer (ALPHA 2-4, Christ, Germany) to obtain CPP powder. The dried CPP were stored in the refrigerator before measurements and treatments.

### 2.3. Operating procedure

Experiments were conducted with CPP solution for the standard stock polysaccharides solution. The standard stock CPP solution of 1.0 mg/ml were prepared by diluting the corresponding mass of dried CPP in ultra pure water. The standard working CPP solution were prepared by further dilution of standard stock CPP solution. The calibration curves were established by measuring a series of the solutions (0.25, 0.5, 0.75, and 1.0 mg/ml). A blank signal was obtained using distilled water. Twenty millilitres of the solution was placed in a cylindrical glass vessel. Effects of initial concentration of CPP solution (0.25, 0.5, 0.75, and 1.0 mg/ml), H<sub>2</sub>O<sub>2</sub> dosages (0.156, 0.312, 0.623, 0.935 and 1.246 mM), solution pH (4, 5, 6, 7, 8, 9 and 10), temperature (20, 30, 40, 50 and 60 °C) and six contact time (10, 20, 30, 40, 50 and 60 min) employed as well. Samples were withdrawn from the sampling port at regular certain reaction intervals, about 1.5 ml of sample was withdrawn, analyzed with a double beam UV–visible spectrophotometer (TU-1900, PGENENAL, Beijing, China), and returned quickly back to the vessel.

All the measurements were made in replicates, standard deviation and relative standard deviation were calculated, which means that measurements were kept under continuous statistical control. An ultrasonic bath (KQ-50E, Kunming Ultrasonic Instrument Co., Kunming, China) with a constant frequency of 40 kHz and at sonic power of 100 W, and with temperature can be changed from 10 to 80 °C was used in this experiment. The temperature of the solution was modulated by this processor, and the pH of the solution was measured by using digital pH meter (Shanghai Precision & Scientific Instrument Co., Shanghai, China). pH adjustments were made by addition of HCl or NH<sub>4</sub>OH to the CPP stock solution in the range of 4–10 (25 °C).

### 2.4. Decolorization measurement

The decolorization of CPP were measured according to the method described by Wang, Qin, Guo, Wang, and Zheng (2005) with a double beam UV–visible spectrophotometer at a wavelength of 420 nm. Decolorization efficiency was calculated by comparing the absorbance value of the CPP solution after treatment to the absorbance value of the original CPP solution. A blank signal was obtained using ultra pure water. The decolorization efficiency was defined as (Carabasa, Ibarz, Garza, & Barbosa-Canovas, 1998):

$$\text{Decolorization efficiency (\%)} = \frac{100(A - B)}{A} \quad (1)$$

where *A* is the initial absorbance of CPP solution, *B* is the absorbance of CPP solution after ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation treatment.

### 2.5. HPLC analysis

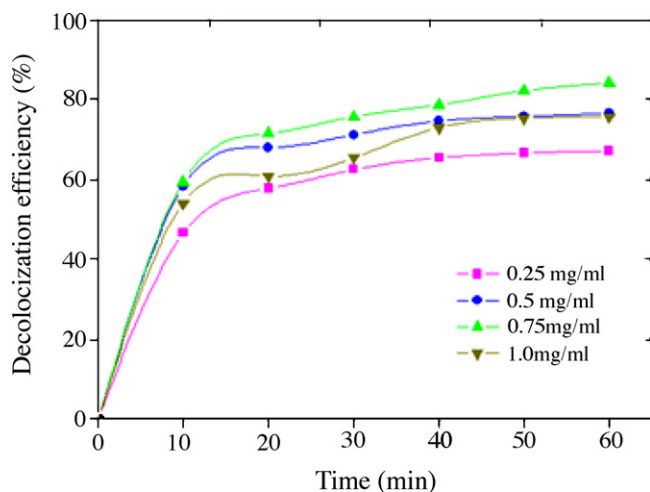
HPLC method (Chen, Xie, Wang, Nie, & Li, 2009) was employed for the measurement of CPP before and after decolorized. The dried hydrolysed glycoprotein and monosaccharide standards were directly labelled with PMP according to the method of Fu and Oneill (1995). Analysis of the PMP-labelled monosaccharide was carried out on a Waters HPLC with a photodiode array detector (PAD) using a special C18 column (250 × 4.6 mm i.d.) that was stable to alkaline solvents. The flow rate was set to 1.0 ml/min at 30 °C and the wavelength for UV detection was 250 nm. The mobile phase comprised 0.1 M PBS solution (pH 6.9) containing 15% (solvent A) and 40% (solvent B) acetonitrile, and the following gradient elution programme was used: 0–10 min, 10–20% A; 10–20 min, 20% A; 20–25 min, 20–10% A; 25–35 min, 10–30% A; 35–40 min, 30–10% A. The injection volume was 20 µl.

### 2.6. Fourier-transformed infrared spectroscopy

FT-IR measurements (FT-IR-5700, Nicolet, USA) were made with diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) equipped with an OMNIC workstation (FTIR, Nicolet, USA) at room temperature. FT-IR spectra were recorded in the range 4000–400 cm<sup>−1</sup> (Wu, Cui, Tang, Wang, & Gu, 2007), at a resolution of 4 cm<sup>−1</sup> and with 200 scans per sample, after preparing a KBr disk containing approximately 2 mg of polysaccharides. The polysaccharides samples were diluted at a 1:50 ratio with KBr powder. A background spectrum was subtracted automatically from the spectrum of each sample.

### 2.7. NMR spectroscopy

For NMR measurements CPP was dried in a vacuum over P<sub>2</sub>O<sub>5</sub> for several days, and then exchanged with deuterium by lyophilizing with D<sub>2</sub>O for several times (Duenas-Chasco et al., 1997). The deuterium-exchanged polysaccharide sample was put in a 5-mm NMR tube and dissolved in 99.9% D<sub>2</sub>O. Spectra were recorded at



**Fig. 1.** Effect of the initial polysaccharides concentration on the decolorization efficiency of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process ( $\text{H}_2\text{O}_2$ , 0.623 mM; pH, 9.0; temperature, 40 °C; contact time, 60 min).

50 °C, using Bruker DRX-400 NMR spectrometer (Bruker, Rheinstetten, Germany), operating at 400.15 MHz for protons, equipped with pulse gradient units, capable of producing magnetic field pulsed gradients in the z-direction of 50 G/cm.

### 3. Results and discussion

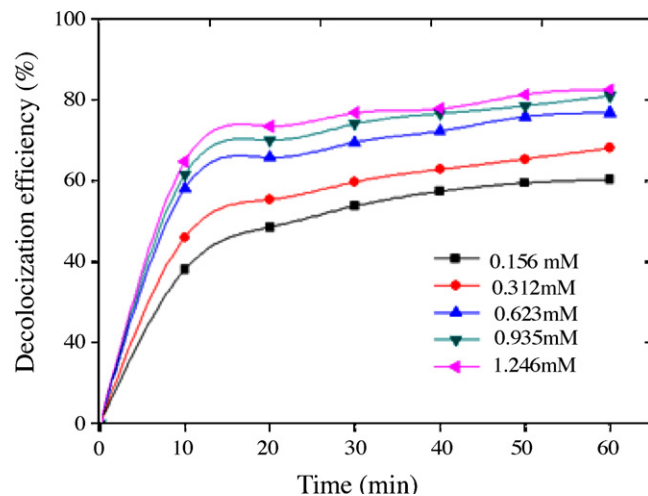
#### 3.1. Effect of initial polysaccharides concentration

The effect of the initial concentration of CPP on the decolorization efficiency was shown in Fig. 1.

As seen in the figure, decolorization efficiency of CPP was decreased as the initial CPP concentration increased. This negative effect can be explained by considering that, the  $\cdot\text{OH}$  free radicals reach equilibrium with the concentration of  $\text{H}_2\text{O}_2$ , as the concentration of CPP increased, the equilibrium adsorption of  $\cdot\text{OH}$  on the CPP active sites increases, hence competitive adsorption of  $\cdot\text{OH}$  on the same sites decreases, the fewer  $\cdot\text{OH}$  available are required to oxidize more molecules, which means a lower formation rate of  $\cdot\text{OH}$  radical which is the principal oxidant indispensable for a high degradation efficiency. Reversely, decreasing the concentration of CPP to 0.25 mg/ml caused a deduction effect. The results indicated that at an appropriate initial concentration of CPP, high decolorization efficiency can also be achieved about 80% at 60 min. Fig. 1 also showed the effect of contact time on the decolorization efficiency of CPP. The results plotted in the figure also suggested that the decolorization efficiency increased with contact time. The increase in decolorization efficiency of CPP was significant in 10 min. Meanwhile, it could be observed that at 0.5 mg/ml of CPP, the decolorization efficiency of the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process was implemented significantly. Therefore, 0.5 mg/ml of CPP was found to be the optimal concentration in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process.

#### 3.2. Effect of the $\text{H}_2\text{O}_2$ dosages

The initial dosages of  $\text{H}_2\text{O}_2$  is an important parameter for the decolorization of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process. The effect of initial dosages of  $\text{H}_2\text{O}_2$  on the decolorization efficiency of CPP was showed in Fig. 2. The results showed that the decolorization of CPP was increased by increasing the dosages of  $\text{H}_2\text{O}_2$ . Meanwhile, the results showed that the 50.3% decolorization of CPP could be reached within 10 min, while  $\text{H}_2\text{O}_2$  dosages varied from 0.156 to 1.246 mM. Theoretically, the higher decolorization



**Fig. 2.** Effect of the dosages of  $\text{H}_2\text{O}_2$  on the decolorization efficiency of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process (CPP concentration, 1.0 mg/ml; pH, 9.0; temperature, 40 °C; contact time, 60 min).

efficiency in the higher dosages of  $\text{H}_2\text{O}_2$  is in the result of faster formation of  $\cdot\text{OH}$  free radical (Modirshahla, Behnajady, & Ghanbary, 2006). However, the increase in decolorization efficiency of CPP was not significant when the dosages of  $\text{H}_2\text{O}_2$  were above 0.935 mM  $\text{H}_2\text{O}_2$ . The results indicated that the concentration of  $\cdot\text{OH}$  radical at the 0.623 mM level was adequate for the oxidation reaction. This could be due to that the solutions were saturated with  $\cdot\text{OH}$  radicals at the 0.623 mM  $\text{H}_2\text{O}_2$  dosage level, and recombination of  $\cdot\text{OH}$  radicals and also  $\cdot\text{OH}$  radicals reacted with  $\text{H}_2\text{O}_2$ , contributing to the  $\cdot\text{OH}$  scavenging capacity (Eqs. (2–4)) (Ghaly, Hartel, Mayer, & Haseneder, 2001).



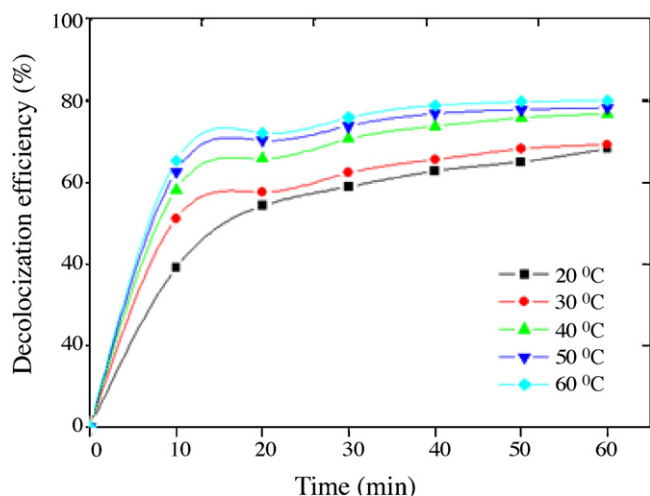
It can be postulated that  $\text{H}_2\text{O}_2$  should be added at an optimum concentration to achieve the best decolorization, hence with a dose of 0.623 mM  $\text{H}_2\text{O}_2$  appear to be an optimum dosage for 1.0 mg/ml of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process.

#### 3.3. Effect of temperature

One of the important parameters that could affect the decolorization efficiency of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  process was temperature. The effect of operating temperature on the decolorization of CPP was carried out at 20, 30, 40, 50 and 60 °C, respectively, while the other variables were kept constant ( $\text{H}_2\text{O}_2$ , 0.623 mM, pH 9.0; initial concentration of CPP, 1.0 mg/ml). As seen in Fig. 3, the effect of temperature on decolorization efficiency of CPP was pronounced, and their color removals increased with the increase of the temperature. It was observed that the decolorization efficiency of CPP increased from 38.1% to 65.3% with temperature from 20 to 60 °C at 10 min. Although the increase of temperature benefits to the decolorization efficiency, both the investment and operational costs are high in the actual treatment of polysaccharides. More than 80% of the decolorization efficiency were achieved at 40 °C.

#### 3.4. Effect of pH

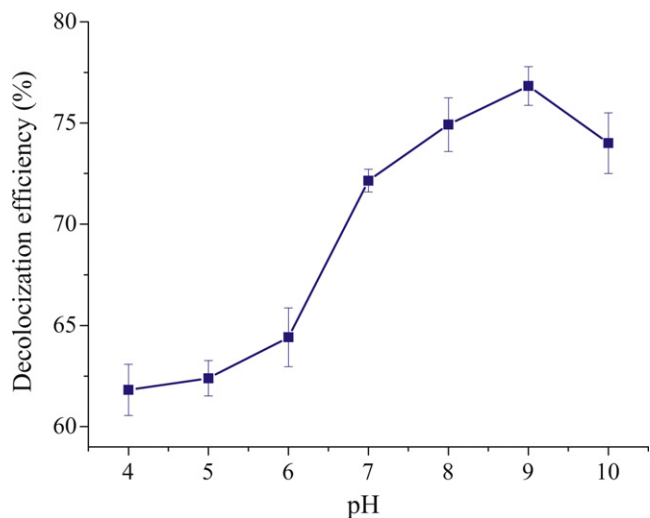
The decolorization of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process were highly influenced by the pH of the solution. To examine the effect of initial pH on the decolorization efficiency of CPP, the



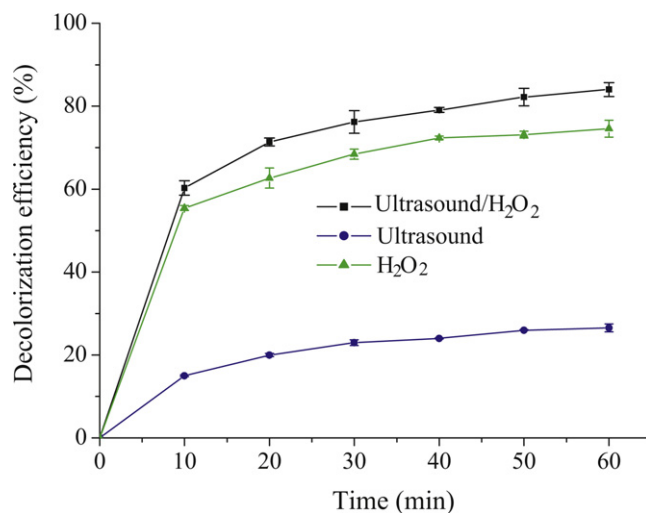
**Fig. 3.** Effect of temperature on the decolorization efficiency of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process ( $\text{H}_2\text{O}_2$ , 0.623 mM; pH, 9.0; CPP concentration, 1.0 mg/ml; contact time, 60 min).

CPP solution were performed at pHs ranging from 4 to 10 with an initial CPP concentration of 1.0 mg/ml, also the fixed concentration ratio of  $\text{H}_2\text{O}_2$  to CPP solution of 1 ml, temperature at 40 °C, and contact time 60 min employed as well. The effect of pH values on the decolorization efficiency of CPP was shown in Fig. 4.

As seen in Fig. 4, the decolorization efficiency of CPP was found to be highest at pH 9, which was in agreement with the studies of dyes decolorization (Sanghi, Bhattacharya, & Singh, 2006). After 40 min, the decolorization efficiency increased from 60.2% to 77.1% as a consequence of increasing pH of the solution from 4 to 9. On the other hand, the decolorization efficiency decreased from 77.1% to 69.9% with increasing pH value of the solution from 9 to 10 (Fig. 4). This may be due to the pH value influences the generation of hydroxyl radicals, thus influences the decolorization efficiency. At a pH below 7, the amount of hydroxyl radicals would decrease, therefore, as a result the decolorization efficiency decreases, while, at a pH above 9, decolorization efficiency decreases due to the oxidation potential of hydroxyl radical was known to decrease with increasing pH (Tadolini & Cabrini, 1990). In this study, a maximum decolorization of 77.1% at 60 min was obtained at pH 9. Therefore, pH 9 was found to be the optimal pH.



**Fig. 4.** Effect of pH on the decolorization efficiency of CPP in the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process ( $\text{H}_2\text{O}_2$ , 0.623 mM; temperature, 40 °C; CPP concentration, 1.0 mg/ml; contact time, 60 min).



**Fig. 5.** The comparison of different decolorization techniques of using ultrasound,  $\text{H}_2\text{O}_2$  and ultrasound/ $\text{H}_2\text{O}_2$  on the decolorization efficiency of CPP (CPP concentration, 0.5 mg/ml;  $\text{H}_2\text{O}_2$ , 0.623 mM; temperature, 40 °C; contact time, 60 min).

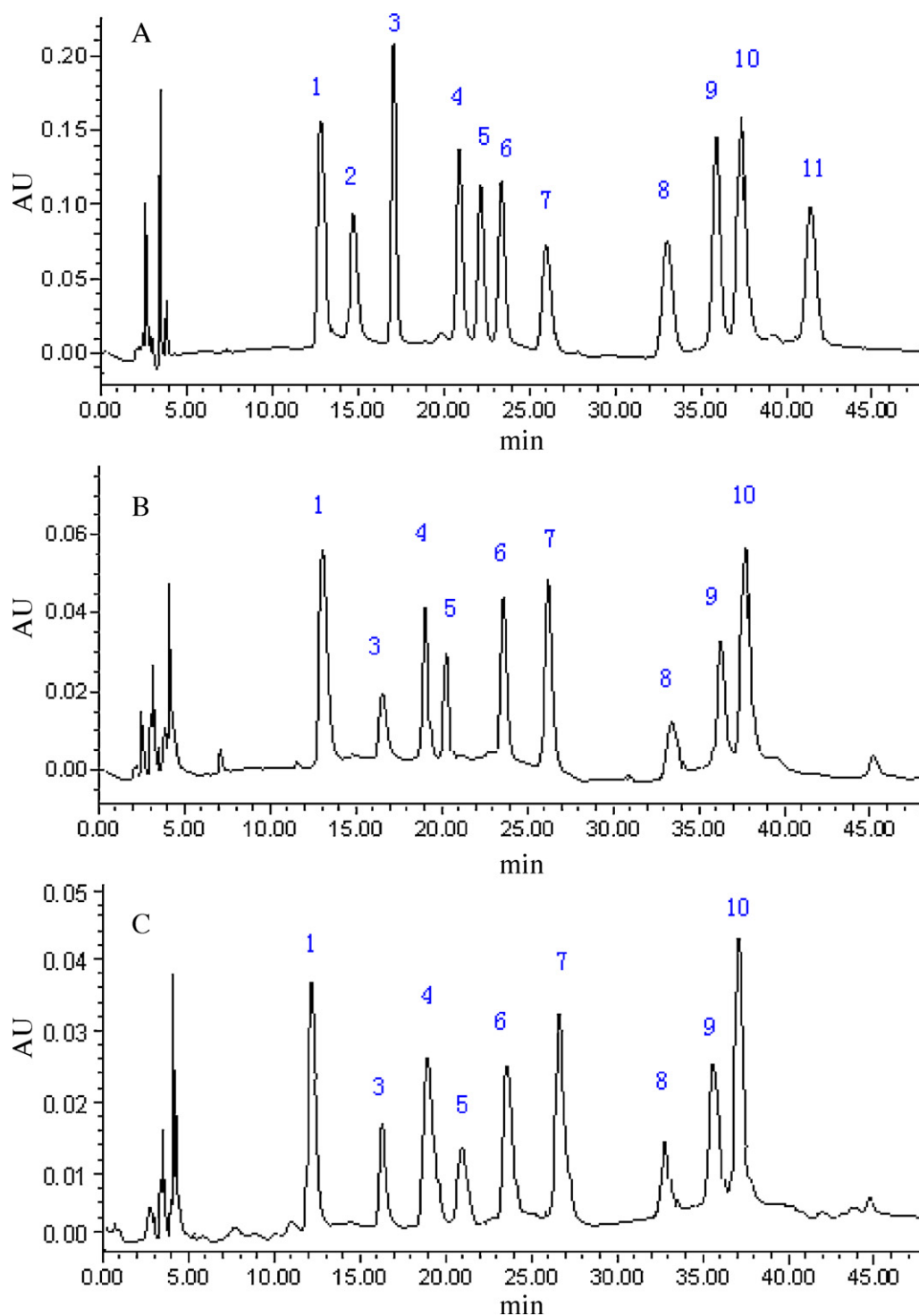
### 3.5. Comparison of different decolorization techniques

In the experiments we describe a comparative study of the decolorization techniques of using ultrasound,  $\text{H}_2\text{O}_2$  and ultrasound/ $\text{H}_2\text{O}_2$ . Fig. 5 showed the decolorization efficiency of CPP versus time for different processes. As seen in Fig. 5, it was evident that the fastest decolorization of CPP was obtained by ultrasound/ $\text{H}_2\text{O}_2$  oxidation process with 77.3% decolorization efficiency. Decolorization of CPP with  $\text{H}_2\text{O}_2$  oxidation process was also observed to be efficient (71.2%). In contrast, the ultrasound radiation treatments were found to be less efficient, and the decolorization was nearly negligible, with 29.4% decolorization efficiency of CPP. So it can be concluded that CPP could be decolorized in a relatively high reaction rate in ultrasound/ $\text{H}_2\text{O}_2$  process. This could be attributed to the increase in hydroxyl radical generation from coupling of ultrasound irradiation and  $\text{H}_2\text{O}_2$ . Ultrasound irradiation plays an important role in the formation of plentiful  $\cdot\text{OH}$  in the solution (Mark et al., 1998). At the presence of ultrasound irradiation, more hydroxyl radicals were produced upon photodissociation of  $\text{H}_2\text{O}_2$ , hence decolorization efficiency of CPP increased. The application of ultrasound/ $\text{H}_2\text{O}_2$  process obtained a higher decolorization efficiency, and required less time when compared to ultrasound and  $\text{H}_2\text{O}_2$ . The results suggested that the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process was a suitable approach for the decolorization of CPP.

### 3.6. HPLC, FT-IR and NMR analysis

The polysaccharides from *C. paliurus* were analyzed using high performance liquid chromatography. Peaks in the obtained chromatograms were identified by comparing the retention time and on-line UV spectra with those of the standards (Fig. 6). Through comparing the retention time with standards (Fig. 6A), the monosaccharide composition was identified (Fig. 6B, C). Five monosaccharides and disaccharides, including rhamnose, arabinose, xylose, mannose, glucose and galactose, were identified for polysaccharides from *C. paliurus*. The relative molar percentages of rhamnose, arabinose, xylose, mannose, glucose and galactose in polysaccharides from *C. paliurus* were 10.6%, 30.2%, 4.6%, 8.7%, 31.8% and 14.1%, respectively. The results showed that the monosaccharides of CPP before and after decolorized were close to each other, indicating that the ultrasound/ $\text{H}_2\text{O}_2$  oxidation process had no effect on the monosaccharide components of CPP.





**Fig. 6.** The HPLC chromatograms of PMP derivatives of 10 standard monosaccharides (A), component monosaccharides released from CPP without ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation treatment (B) and component monosaccharides released from CPP with ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation treatment. The HPLC analysis was carried out as described in Section 2.5. Peaks: 1. PMP; 2. ribose; 3. mannose; 4. rhamnose; 5. glucuronic acid; 6. galacturonic acid; 7. glucose; 8. xylose; 9. galactose; 10. arabinose; 11. fucose.

FT-IR spectra method was used for the analysis the CPP before and after the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process treatment. The results showed that the FT-IR spectrum of CPP displayed a broad stretching intense peak at around 3420 cm<sup>-1</sup> characteristic for hydroxyl and amine groups, and a weak C–H stretching band at 2923 cm<sup>-1</sup>. The peak around 2121 cm<sup>-1</sup> also indicated aliphatic C–H bonds. Fig. 7 showed that the bands in the region of 1646 cm<sup>-1</sup> was principally meant carboxyl group existed (Santhiya, Subramanian, & Natarajan, 2002). Further, it can be found that

two stretching peaks at 1154 cm<sup>-1</sup> and 1077 cm<sup>-1</sup> suggested the presence of C–O bond. The spectrum of a glycosidic bond appeared at approximately 915 cm<sup>-1</sup> suggested that CPP was composed of sugar derivatives (Zhou, Zhang, Yao, Niu, & Gao, 2010). A band of absorption at 773 cm<sup>-1</sup> represented symmetrical ring vibration. This typical feature of CPP spectra suggested that the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process under the condition given did not result in any significant change in the structure of CPP.

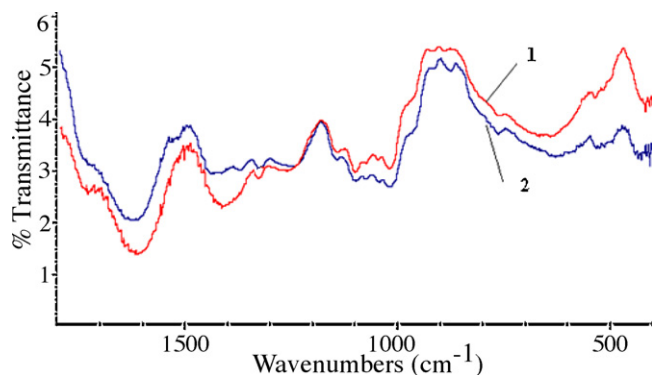


Fig. 7. FT-IR spectra of CPP (1. without treatment; 2. after ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process treatment).

The <sup>1</sup>H NMR spectrum of CPP before and after decolorized was illustrated in Fig. 8. From the figure about the comparison of group A and B, we can see that they are basically the same. Signals at 5.10, 3.82, 3.99, and 4.38 ppm were assigned to H-1 to H-4 of (1→4) α-galacturonic acid residues, respectively (Hokputsa et al., 2004). Obviously, a strong signal at 4.7 ppm in Fig. 8 was originated from the residual solvent (HDO) (Bian, Peng, Xu, Sun, & Kennedy, 2010). The chemical shift at 5.02 ppm of anomeric H-1 indicated a form of L-arabinofuranosyl unit (Yang et al., 2009). The signal at 1.19 ppm was assigned to the CH<sub>3</sub> of α-L-rhamnopyranose units. The proton signals near 2.1 ppm arise from the –CH<sub>3</sub> of the acetyl groups (Sun, Cui, Tang, & Gu, 2010). These results suggested that the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process had no significant effect on the structure of CPP.

#### 4. Conclusions

From the results of this work, it can be concluded that the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process provides good performance in the decolorization treatment of CPP. The decolorization efficiency of CPP was affected significantly by the initial concentration of CPP solution, dosages of H<sub>2</sub>O<sub>2</sub>, temperature and pH. In general, increasing the dosages of H<sub>2</sub>O<sub>2</sub> increased the decolorization efficiency of polysaccharides, but there was an optimum value of dosage, over which the decolorization efficiency was increased slowly. Besides, pH, temperature, and contact time also played important roles in the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation decolorization process of CPP. This investigation also confirmed the presence of ultrasound irradiation could enhance the effectiveness of decolorization. The optimum operational conditions for decolorization were determined as follows: 0.623 mM H<sub>2</sub>O<sub>2</sub>, 0.5 mg/ml of CPP solution, 40 °C, pH=9.0, with a decolorization efficiency of 84.1%. Moreover, the results of HPLC, FT-IR and NMR analysis suggested that the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process under the optimum condition did not result in any significant change in the structure of CPP. The experimental results showed that the ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation process was a suitable method for decolorization of polysaccharides.

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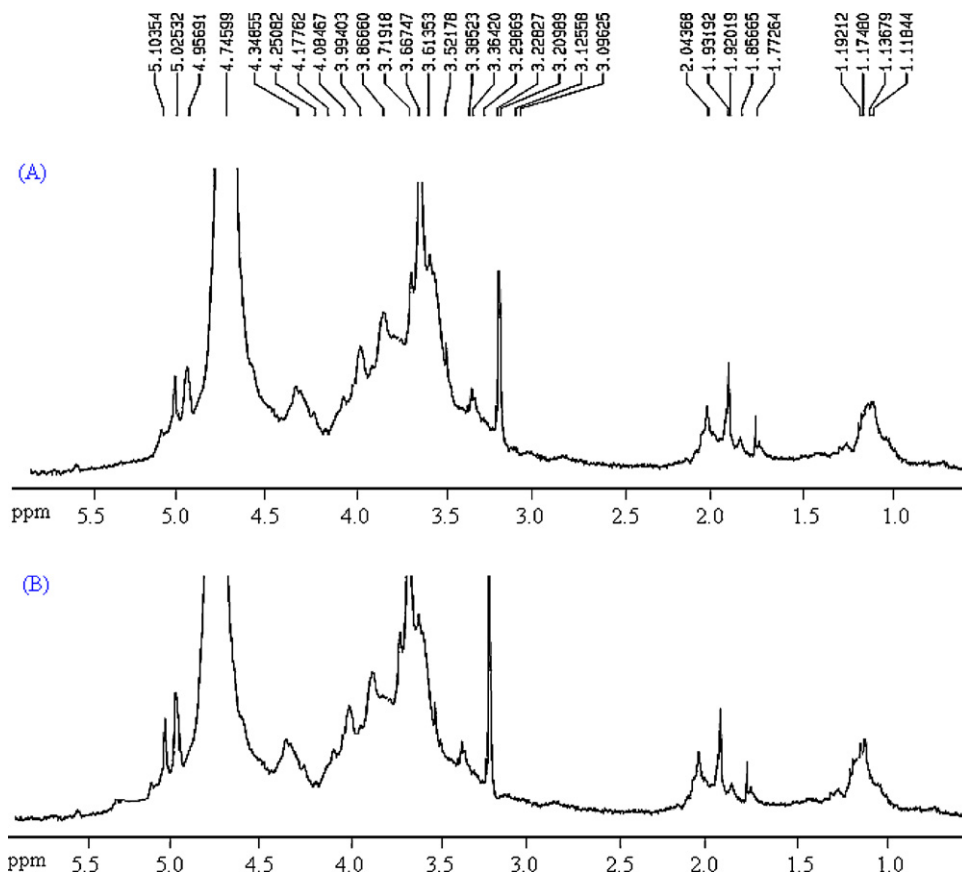


Fig. 8. <sup>1</sup>H NMR analysis of CPP. (A) without treatment; (B) with ultrasound/H<sub>2</sub>O<sub>2</sub> oxidation treatment.

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## References

- Achaerandio, I., Guéll, C., & López, F. (2002). Continuous vinegar decolorization with exchange resins. *Journal of Food Engineering*, 51, 311–317.
- Adewuyi, Y. G. (2001). Sonochemistry: Environmental science and engineering applications. *Industrial & Engineering Chemistry Research*, 40, 4687–4715.
- Ahmedna, M., Marshall, W. E., & Rao, R. M. (2000). Surface properties of granular activated carbons from agricultural by-products and their effects on raw sugar decolorization. *Bioresource Technology*, 71, 103–112.
- Bian, J., Peng, F., Xu, F., Sun, R. C., & Kennedy, J. F. (2010). Fractional isolation and structural characterization of hemicelluloses from *Caragana korshinskii*. *Carbohydrate Polymers*, 80, 753–760.
- Carabasa, M., Ibarz, A., Garza, S., & Barbosa-Canovas, G. V. (1998). Removal of dark compounds from clarified fruit juices by adsorption processes. *Journal of Food Engineering*, 37, 25–41.
- Chen, Y., Xie, M. Y., Wang, Y. X., Nie, S. P., & Li, C. (2009). Analysis of the monosaccharide composition of purified polysaccharides in *Ganoderma atrum* by capillary gas chromatography. *Phytochemical Analysis*, 20, 503–510.
- Duenas-Chasco, M. T., Rodriguez-Carvajal, M. A., Mateo, P. T., Franco-Rodriguez, G., Espartero, J., Irastorza-Iribas, A., et al. (1997). Structural analysis of the exopolysaccharide produced by *Pediococcus damnosus*. *Carbohydrate Research*, 303, 453–458.
- Fang, S. Z., Wang, J. Y., Wei, Z. Y., & Zhu, Z. X. (2006). Methods to break seed dormancy in *Cyclocarya paliurus* (Batal.) Iljinskaja. *Scientia Horticulturae*, 110, 305–309.
- Fu, D. T., & Oneill, R. A. (1995). Monosaccharide composition analysis of oligosaccharides and glycoproteins by high-performance liquid chromatography. *Analytical Biochemistry*, 227, 377–384.
- Ghaly, M. Y., Hartel, G., Mayer, R., & Haseneder, R. (2001). Photochemical oxidation of p-chlorophenol by UV/H<sub>2</sub>O<sub>2</sub> and photo-Fenton process. A comparative study. *Waste Management*, 21, 41–47.
- Hokputsa, S., Gerddit, W., Pongsamart, S., Inngjerdigen, K., Heinze, T., Koschella, A., et al. (2004). Water-soluble polysaccharides with pharmaceutical importance from Durian rinds (*Durio zibethinus* Murr.): Isolation, fractionation, characterisation and bioactivity. *Carbohydrate Polymers*, 56, 471–481.
- Kurihara, H., Asami, S., Shibata, H., Fukami, H., & Tanaka, T. (2003). Hypolipemic effect of *Cyclocarya paliurus* (Batal.) Iljinskaja in lipid-loaded mice. *Biological & Pharmaceutical Bulletin*, 26, 383–385.
- Li, L., Xie, M. Y., Sun, Z. H., Wu, X. H., Sun, D. H., & Wang, X. R. (2000). The study on the element transference characteristics and element speciation in the extract of *Cyclocarya paliurus* (Batal.) Iljinskaja leaves. *Chemical Journal of Chinese Universities-Chinese*, 21, 707–709.
- Liu, X., Wang, S. Q., Xie, M. Y., Xie, J. H., Huang, D. F., & Tang, Y. F. (2007). Effects of polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja on growth of HeLa cells and human umbilical vein endothelial cells. *Food Science*, 28(7), 520–522.
- Mahmoodi, N. M., Arami, M., Limaee, N. Y., & Tabrizi, N. S. (2005). Decolorization and aromatic ring degradation kinetics of Direct Red 80 by UV oxidation in the presence of hydrogen peroxide utilizing TiO<sub>2</sub> as a photocatalyst. *Chemical Engineering Journal*, 112, 191–196.
- Mark, G., Tauber, A., Laupert, R., Schuchmann, H. P., Schulz, D., Mues, A., et al. (1998). OH-radical formation by ultrasound in aqueous solution. Part II. Terephthalate and Fricke dosimetry and the influence of various conditions on the sonolytic yield. *Ultrasonics Sonochemistry*, 5, 41–52.
- Modirshahla, N., Behnajady, M. A., & Ghanbary, F. (2006). Decolorization and mineralization of C.I. acid yellow 23 by Fenton and photo-Fenton processes. *Dyes and Pigments*, 73, 305–310.
- Sanghi, R., Bhattacharya, B., & Singh, V. (2006). Use of Cassia javahikahai seed gum and gum-g-polyacrylamide as coagulant aid for the decolorization of textile dye solutions. *Bioresource Technology*, 97, 1259–1264.
- Santhiya, D., Subramanian, S., & Natarajan, K. A. (2002). Surface chemical studies on sphalerite and galena using extracellular polysaccharide isolated from *Bacillus polymyxa*. *Journal of Colloid and Interface Science*, 256, 237–248.
- Simaratanamongkol, A., & Thiravetyan, P. (2010). Decolorization of melanoidin by activated carbon obtained from bagasse bottom ash. *Journal of Food Engineering*, 96, 14–17.
- Sun, Y. L., Cui, S. W., Tang, J., & Gu, X. H. (2010). Structural features of pectic polysaccharide from *Angelica sinensis* (Oliv.) Diels. *Carbohydrate Polymers*, 80, 544–550.
- Tadolini, B., & Cabrin, L. (1990). The influence of pH on OH scavenger inhibition of damage to deoxyribose by Fenton reaction. *Molecular and Cellular Biochemistry*, 94, 97–104.
- Wang, S. B., Qin, X. M., Guo, X. Q., Wang, Q. L., & Zheng, L. Z. (2005). Decolorization of crude saposinikovia divaricata polysaccharide by resins. *Chinese Journal of Applied Chemistry*, 22, 1308–1311.
- Wu, Y., Cui, S. W., Tang, J., Wang, Q., & Gu, X. H. (2007). Preparation, partial characterization and bioactivity of water-soluble polysaccharides from boat-fruited sterculia seeds. *Carbohydrate Polymers*, 70, 437–443.
- Xie, J. H., & Xie, M. Y. (2008). Review about the research on *Cyclocarya paliurus* (Batal.) Iljinskaja. *Journal of Food Science and Biotechnology*, 27, 113–121.
- Xie, J. H., Xie, M. Y., Nie, S. P., Shen, M. Y., Wang, Y. X., & Li, C. (2010). Isolation, chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja. *Food Chemistry*, 119, 1626–1632.
- Xie, J. H., Xie, M. Y., Shen, M. Y., Nie, S. P., Wang, Y. X., & Li, C. (2010). Optimisation of microwave-assisted extraction of polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja using response surface methodology. *Journal of the Science of Food and Agriculture*, 90, 1353–1360.
- Xie, M. Y., Li, L., Nie, S. P., Wang, X. R., & Lee, F. S. C. (2006). Determination of speciation of elements related to blood sugar in bioactive extracts from *Cyclocarya paliurus* leaves by FIA-ICP-MS. *European Food Research and Technology*, 223, 202–209.
- Yang, B., Jiang, Y. M., Zhao, M. M., Chen, F., Wang, R., Chen, Y. D., et al. (2009). Structural characterisation of polysaccharides purified from longan (*Dimocarpus longan* Lour.) fruit pericarp. *Food Chemistry*, 115, 609–614.
- Zhang, Z. M., & Zheng, H. L. (2009). Optimization for decolorization of azo dye acid green 20 by ultrasound and H<sub>2</sub>O<sub>2</sub> using response surface methodology. *Journal of Hazardous Materials*, 172, 1388–1393.
- Zhou, S., Zhang, X., Yao, W., Niu, Y., & Gao, X. (2010). Structure characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of *Lycium barbarum* L. *Carbohydrate Polymers*, 80, 1161–1167.