The pretreatment effects on the antioxidant activity of jujube polysaccharides

Chenling Qu\textsuperscript{a}, Songcheng Yu\textsuperscript{b}, Huali Jin\textsuperscript{a,*}, Jinshui Wang\textsuperscript{a}, Li Luo\textsuperscript{a}

\textsuperscript{a} College of Grain Oil and Food Science, Henan University of Technology, Zhengzhou 450052, China
\textsuperscript{b} College of Public Health, Zhengzhou University, Zhengzhou 450001, China

HIGHLIGHTS

- Different extraction methods affected the antioxidant activity differently.
- Power was the key parameter in ultrasonic and microwave extraction.
- Powers of 300 W and 600 W were two critical points in microwave extraction.
- Sevag reagent combined with $\text{H}_2\text{O}_2$ was best for protein and pigment removal.

GRAPHICAL ABSTRACT

- OH scavenging activity (%)

A B C D E F G H

ABSTRACT

Pretreatment is vital to keep the bioactivities of polysaccharides. In this paper, the effects of hot water, ultrasonic and microwave extraction, as well as the effects of protein and pigment removal steps, on the antioxidant activity of water soluble polysaccharides in jujube (WSPJ) were studied. Hydroxyl free radical (‘OH) scavenging activity was adopted to determine the antioxidant activity of WSPJ. The results showed that ‘OH scavenging activity of WSPJ extracted by ultrasonic wave was higher than that extracted by hot water and by microwave. Furthermore, power parameter in both ultrasonic and microwave extraction affected the ‘OH scavenging activity dramatically. On the other hand, Sevag reagent was better than trichloroacetic acid (TCA), TCA with 1-butanol (TCA-B) and hydrochloric acid for protein removal, and $\text{H}_2\text{O}_2$ was better than active carbon for pigment removal to keep the antioxidant activity of WSPJ.

Introduction

Plant polysaccharides have attracted more and more attention in recent years due to their potential biological functions, e.g. antioxidant [1,2], antiviral [3], anticoagulant [4], antitumor [5,6], antidiabetes [7], immunological [8,9] and hepatoprotective activities [10]. So far studies on polysaccharides were mainly focused on extraction, purification, structure determination [11,12] and bioactivities.

In addition to hot water extraction [13–15], ultrasonic [16–18] and microwave extractions [19–22] were applied more and more in polysaccharide extraction. The combination of these methods was also used for polysaccharide extractions [23,24].

Ultrasonic and microwave extraction may affect the structures and activities of polysaccharides although they can be more time saving than conventional hot water extraction. In microwave heating process, a sharp decrease in molecular weight of polysaccharides in \textit{Artemisia sphaerocephala} was detected and the degraded...
polysaccharides showed stronger antioxidant activities compared with polysaccharides obtained from hot water extraction [19]. Wang et al. [25] also studied the polysaccharides in *Potentilla anserina* L. after microwave heating and the results were the same as he had reported. Zhou et al. [26] investigated the effects of ultrasonic wave on the structure and antioxidant activity of the polysaccharides in *Porphyra yezoensis* Udea. The results ascertained that ultrasonic degradation did not change the main structure of polysaccharides in the test conditions, and the degraded polysaccharides showed enhanced antioxidant activity. Traditional water extraction (TWE), microwave assisted water extraction (MAE) and ultrasound assisted extraction (UAE) of tea flower polysaccharides were compared by Wei’s group [27]. The results showed that TWE was found to be the optimal method with highest yield and highest neutral and acid saccharides contents, and the polysaccharides obtained by MAE and UAE generally had lower inhibitory effects on α-glucosidase than that obtained by TWE. Before determining the structures of polysaccharides, protein and pigment in crude polysaccharides should be removed. The commonly used protein removal reagents were TCA, TCA-B, hydrochloric acid and Sevag reagent, and pigment removal reagents were hydrogen peroxide \( \text{H}_2\text{O}_2 \) and active carbon. The protein and pigment removal steps can influence the activities of polysaccharides. To find out the best protein and pigment removal reagents to keep the bioactivities of polysaccharides was important. In this paper, WSPJ was taken as the object, and the ‘OH scavenging activity as criterion, to examine the effects of three extraction methods (hot water extraction, ultrasonic extraction and microwave extraction), four protein removal reagents (TCA, TCA-B, hydrochloric acid and Sevag methods) and two pigment removal reagents \( \text{H}_2\text{O}_2 \) and active carbon.

**Experimental**

**Chemicals and instruments**

Jujube (*Ziziphus jujuba* Mill.) was purchased from a local shop, Zhengzhou, China. These jujubes originally grew in Xinjiang province, China. All reagents were analytical purity. Anhydrous ethanol, 95% ethanol and acetone were obtained from Tianli Corporation (Tianjin, China). FeSO\(_4\) (Ferrous sulfate), salicylic acid, NaH\(_2\)PO\(_4\) (sodium dihydrogen phosphate), Na\(_2\)HPO\(_4\) (disodium hydrogen phosphate), K\(_2\)Fe(CN)\(_6\) (potassium ferricyanide), FeCl\(_3\) (iron trichloride), trichloroacetic acid (TCA), 1-butanol, chloroform and petroleum ether were purchased from Kernel Corporation (Tianjin, China). H\(_2\)O\(_2\) (Hydrogen peroxide), HCl (hydrochloric acid), NaOH (sodium hydroxide) and active carbon were obtained from Haohua Corporation (Luoyang, China). Water used in the experiments was purified by Milli-Q system (Millipore Corporation, USA).

KQ5200DE ultrasonic instrument, which control temperature, power and time, was supplied by Kunshan Corporation (Shanghai, China). M68 microwave extraction apparatus was obtained from Meicheng Corporation (Beijing, China). RE-52A rotary evaporation instrument (Yarong Corporation, Shanghai, China) and 752 Spectrophotometer (Jinghua Corporation, Shanghai, China) were also employed in the experiments.

**Procedures**

All the experiment conditions were optimized based on ‘OH scavenging activity. Each experiment was repeated 3 times and the average of the data was adopted.

**Hot water extraction of WSPJ**

Weighed 5.0 g degreased powder (the pulp powder of jujube was refluxed by petroleum ether at 40 °C for 5 h) of jujube, fixed the ratio of the powder to water at 1:20, and optimized extraction temperature (40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C) and extraction time (1 h, 2 h, 3 h, 4 h, 5 h). Then the extraction solution was centrifuged for 15 min at 4000 r min\(^{-1}\). The supernatant was collected and concentrated by rotary evaporator, and the concentrate was deposited by 100 mL 95% ethanol and placed in the refrigerator at 4 °C for 12 h. After that, the obtained mixture was filtrated and the sediment was washed by 50 mL 95% ethanol, 50 mL anhydrous ethanol and 50 mL acetone successively. The washed sediment, which is the WSPJ, was dried at 40 °C.

**Ultrasonic extraction of WSPJ**

Weighed 5.0 g degreased powder of jujube, fixed the ratio of the powder to water at 1:20, and optimized microwave power (200 W, 300 W, 400 W, 500 W, 600 W, 700 W) and extraction time (1 min, 2 min, 3 min, 4 min, 5 min, 6 min). The following steps were the same as the steps in 2.2.1.

**Microwave extraction of WSPJ**

Weighed 5.0 g degreased powder of jujube, fixed the ratio of the powder to water at 1:20, and optimized microwave power (200 W, 300 W, 400 W, 500 W, 600 W, 700 W) and extraction time (1 min, 2 min, 3 min, 4 min, 5 min, 6 min). The following steps were the same as the steps in 2.2.1.

**Scavenging activity (%) = (1 – A\(_{\text{sample}}\)/A\(_{\text{control}}\) \times 100**

**Protein removal by TCA-B**

5% TCA solution was prepared with 1-butanol. Then 50 mL TCA-B solution was added into 50 mL 4 mg mL\(^{-1}\) polysaccharides water solution in a separatory funnel. After shaking and separating, the subnatant was collected and neutralized by 2 mol L\(^{-1}\) NaOH, then precipitated by 100 mL 95% ethanol. The mixed was placed in the refrigerator at 4 °C for 12 h. Then the precipitate was washed by 50 mL 95% ethanol, 50 mL anhydrous ethanol and 50 mL acetone orderly. The washed precipitate was dried at 40 °C, and the protein-free WSPJ was obtained.

**Protein removal by TCA**

10% TCA was added to 50 mL 4 mg mL\(^{-1}\) WSPJ to pH 3.0, and the solution was kept stand for 12 h. Then the solution was centrifuged for 10 min (3000 r min\(^{-1}\)) and the supernatant was precipitated by 100 mL 95% ethanol at 4 °C for 12 h. Then the precipitate was treated by 95% ethanol, anhydrous ethanol, acetone and dried as steps in 2.2.5.

**Protein removal by hydrochloric acid**

2 mol L\(^{-1}\) HCl was added to 50 mL 4 mg mL\(^{-1}\) WSPJ to pH 3.0, and the solution was kept stand for 12 h. The solution was centrifuged, and the supernatant was precipitated and the precipitate was washed and dried as steps in 2.2.6.

**Protein removal by Sevag reagent**

17 mL Sevag reagent \((V_{\text{chloroform}}: V_{\text{1-butanol}} = 4:1)\) was added to 50 mL 4 mg mL\(^{-1}\) WSPJ and shaked. The solution was kept stand
for 30 min, and the subnatant was discarded. The above steps were repeated 4 times. Then the precipitate was washed and the precipitate was dried as steps in 2.2.6.

**Pigment removal by active carbon**

3 mg active carbon powder was added to 50 mL 4 mg mL$^{-1}$ WSPJ and the mixture was put into 80 °C water bath for 30 min. Then the mixture was filtrated and the liquid was precipitated by 95% ethanol at 4 °C for 12 h. Then the precipitate was washed and dried as steps in 2.2.6. The pigment-free polysaccharides were obtained.

**Pigment removal by H$_2$O$_2$**

20 mL 10% H$_2$O$_2$ was added to 50 mL 4 mg mL$^{-1}$ WSPJ and the solution was put into 60 °C water bath for 4 h. Then the solution was precipitated by 95% ethanol at 4 °C for 12 h. Then the precipitate was washed and dried at 40 °C.

**Results and discussion**

**Optimization of hot water extraction**

The time and temperature of hot water extraction were optimized, and the results were shown in Fig. 1. It can be seen from Fig. 1(A) that the ·OH scavenging activity went up and then dropped versus extraction time and the optimal extraction time...
was 3 h. When the extraction time was longer than 3 h, the activity of WSPJ fell down. The extraction temperature had little effect on ·OH scavenging activity when the temperature was lower than 80 °C, shown in Fig. 1(B). But the activity dropped obviously when the temperature was higher than 80 °C. It can be concluded that long time extraction and high temperature extraction could destroy the antioxidant activity of the WSPJ.

**Optimization of ultrasonic extraction**

It can be seen from Fig. 2 that the optimal extraction time, extraction temperature and ultrasonic power in the ultrasonic extraction were 15 min, 40 °C and 80 W, respectively. The data in Fig. 2(A) showed that extraction time did not affect the ·OH scavenging activity of WSPJ obviously. The ·OH scavenging activity of WSPJ reached maximum when extraction temperature was 40 °C and reduced a little and remained stable when the temperature was between 50 °C and 80 °C Fig. 2(B). However, the ultrasonic power influenced the ·OH scavenging activity seriously. The activity dropped a lot and rapidly when the ultrasonic power was from 80 W to 120 W, and the activity remained almost unchanged when the ultrasonic power was from 120 W to 180 W Fig. 2(C). These results indicated that ultrasonic power was the key parameter in ultrasonic extraction. The reason may be that high ultrasonic power could partly destroy the active structure of WSPJ during the extraction.

**Optimization of microwave extraction**

The effects of microwave extraction time and microwave power on the ·OH scavenging activity of WSPJ were shown in Fig. 3. The optimal microwave extraction time and microwave power were 4 min and 300 W, respectively. Fig. 3(A) displays that ·OH scavenging activity increased along with extraction time and almost remained stable after 4 min extraction. On the other hand, microwave power affected the ·OH scavenging activity significantly Fig. 3(B). The ·OH scavenging activity decreased along with microwave power from the whole view. The reduction could be seen as two stages. The first one was from 200 W to 500 W. The activity changed a little from 200 W to 300 W, and dropped obviously from 300 W to 500 W. In the other one, from 500 W to 700 W, the activity changed a little from 500 W to 600 W, and dropped sharply from 600 W to 700 W. The results indicated that, in different power range, microwave could destroy the active structure of WSPJ to different extent. This suggested that partial active structure of WSPJ could tolerate no more than 300 W, that partial active structure could tolerate no more than 600 W, and that, when the power was larger than 600 W, the active structure was destroyed dramatically.

![Fig. 3](image.png)

**Fig. 3.** (A) Optimization microwave extraction time when microwave power was 400 W. (B) Optimization microwave power when extraction time was 4 min.

**Table 1**

<table>
<thead>
<tr>
<th>Extraction method (optimal condition)</th>
<th>·OH scavenging activity of WSPJ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water extraction (3 h, 60 °C)</td>
<td>54</td>
</tr>
<tr>
<td>Ultrasonic extraction (15 min, 40 °C, 80 W)</td>
<td>68</td>
</tr>
<tr>
<td>Microwave extraction (4 min, 300 W)</td>
<td>52</td>
</tr>
</tbody>
</table>

![Fig. 4](image.png)

**Fig. 4.** The comparison of the effects of different protein and pigment removing methods on the ·OH scavenging activity of WSPJ (·OH scavenging activity of crude WSPJ extracted by hot water was defined as 100%). (A) The crude WSPJ obtained from hot water extraction. (B) The polysaccharides obtained from (A) by removing proteins with TCA-B. (C) The polysaccharides obtained from (A) by removing proteins with TCA. (D) The polysaccharides obtained from (A) by removing proteins with hydrochloric acid. (E) The polysaccharides obtained from (A) by removing proteins with Sevag reagent. (F) The polysaccharides obtained from (A) by removing pigment with carbon carbon. (G) The polysaccharides obtained from (A) by removing pigment with H2O2. (H) The polysaccharides obtained from (A) by removing proteins with Sevag reagent and pigment with H2O2.
Comparison of different polysaccharides extraction methods

It can be seen from Table 1 that the ‘OH scavenging activity under each optimal condition of hot water extraction, ultrasonic extraction and microwave extraction was 54%, 68% and 52%, respectively. The ‘OH scavenging activities extracted by hot water and microwave almost the same, and that extracted by ultrasonic was highest. In addition, hot water extraction was time-consuming, and microwave extraction and ultrasonic extraction cost only 5 min and 15 min, respectively. Therefore, considering the antioxidant activity and the extraction time, ultrasonic extraction was the best.

Comparison of different protein and pigment removal methods

The comparison results of different protein and pigment removal methods were shown in Fig. 4. The data showed that for protein removal, Sevag reagent had the minimal damage to the ‘OH scavenging activity, and H$_2$O$_2$ was better than active carbon for pigment removal. So Sevag reagent and H$_2$O$_2$ reagent can be combined to eliminate proteins and pigments to maintain maximum antioxidant activity of polysaccharides.

The results showed that all these four protein removal reagents and two pigment removal reagents had negative impacts on the ‘OH scavenging activity of WSPJ. The reason may be that these treatments had effects on the active structures of WSPJ. On the other hand, the glycoprotein in jujube perhaps had ‘OH scavenging activity and they were destroyed during these treatments. Therefore, the reagents which had least damages to the antioxidant activity of WSPJ should be chosen for protein and pigment removal.

Conclusion

In this paper, the effects of three extraction methods, four protein removal methods and two pigment removal methods on the antioxidant activity of WSPJ were investigated. The results suggested that the extraction conditions should be well controlled especially power parameter in both ultrasonic and microwave extraction. The results also indicated that Sevag reagent was best for protein removal and that H$_2$O$_2$ was fit for pigment removal.

Acknowledgement

This research was supported by Grants from the Doctor Research Fund of Henan University of Technology (No. 2009BS027).