

Research Article

Activity and Structural Characteristics of Peach Gum Exudates

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Polysaccharide fractions were prepared from peach gum exudates by treatments with alkaline hydrogen peroxide (AHP) and liquid hot water (LHW). The structural characteristics and activities of the polysaccharide fractions were comparatively studied. The results suggested that arabinogalactans substituted with xylose and uronic acids were the main structure of all polysaccharide fractions. AHP and LHW treatments introduced the degradation of the polysaccharides, reducing the molecular weight of polysaccharides from 182500 g/mol to 78450 g/mol and 68420 g/mol, respectively. The decrease of molecular weights responded to the decrease of thermal stability of polysaccharide fractions. However, AHP and LHW treatments removed most of the nonsugar composition, increasing the DPPH[•]- and ABTS^{•+}-scavenging activity of polysaccharides. Polysaccharide fractions obtained from LHW treatment showed favorable DPPH[•]- and ABTS^{•+}-scavenging activity at 22.9% and 34.3%, respectively, at concentration of 1000 µg/mL.

1. Introduction

Peach gum, produced from the fruit and trunk of peach tree in response of mechanical injury or microbial invasion, is composed of polysaccharides with complex structures [1–4]. Stephen suggested that the polysaccharide components of the gum from peach tree belonged to the arabinogalactan group with amounts of arabinose, xylose, galactose, glucose, and uronic acids [5]. Due to the large number of monosaccharide, most of the gum polysaccharides have a great number of glycosidic linkages, highly branched structure and with a high molecular weight. However, these features of gum polysaccharides lead to a low solubility in water, limiting its wide application, particularly in medicine and food industry. In order to improve the solubility and physicochemical properties of the gum polysaccharides, some treatments are used to prepare water-soluble polysaccharides [6, 7].

Liquid hot water treatment is one of the effective configurations for cleaving the linkages between sugars. At high temperature, protons released from hot water can catalyze

the breakage of linkages in the substitutes and even a backbone, making a hydrolysis of polysaccharides. The proton also can cleave the uronic acids and acetyl units thus liberating acids which facilitate the degradation of polysaccharides. However, the sugars may further degrade by liquid hot water into furfural and 5-hydroxymethyl furfural. Hydrogen peroxide is easy to handle, available, and environmentally friendly. It has the ability of the formation of free radicals, which can attack the linkages of the polysaccharides. Based on this theory, hydrogen peroxide is used to hydrolyze polysaccharides, such as chitosan [8] and peach gum [9].

In this investigation, alkaline hydrogen peroxide (AHP) and liquid hot water (LHW) were adopted to treat the gum exudates to obtain water soluble polysaccharides. Structural features, physicochemical properties, and antioxidant activities of the obtained polysaccharide fractions were comparatively studied. The complete insight into the characteristics of peach gum polysaccharides is essential for its application in foods and other products.

2. Materials and Methods

2.1. Materials and Fractionation of Polysaccharides. Peach gum was collected from the trunk of peach tree (*Prunus persica*) (Naxi, Lijiang, Yunnan Province, China) and labeled as raw gum (RG).

The RG was further treated with alkaline hydrogen peroxide (AHP) and liquid hot water (LHW) to obtain water-soluble polysaccharide fractions. The AHP treatment was conducted as 4 g of RG soaked in 200 mL alkaline hydrogen peroxide solution (containing 0.5% (w/v) NaOH and 1% (v/v) H₂O₂) at 50°C for 2 h. The LHW extraction was performed at 140°C at solid to liquid ratio of 1 : 50 for 2 h. At the end of these treatments, the supernatants were collected by centrifugation, adjusted to pH 5.5–6.0 with 6 M HCl, then concentrated to 30 mL using a vacuum rotary evaporator, and poured into 90 mL ethanol. The formed precipitates were the polysaccharide fractions from peach gum. These polysaccharides were recovered by centrifugation, freeze-dried and labeled according to the treatments as P-AHP and P-LHW, respectively. All the samples were kept in a desiccator at room temperature for further analysis. To reduce errors and confirm the results, each experiment was repeated in double under the same condition.

2.2. Analysis Methods. The composition of monomeric sugars and uronic acids were determined using the National Renewable Energy Laboratory (NREL) protocol [10] and analyzed by the high-performance anion exchange chromatography (HPAEC) (Dionex, ICS-3000, USA) system on a CarboPac PA20 (Dionex, USA) according to the literature [11].

The average molecular weights of the samples were performed on an Agilent 1200 HPLC system with a refractive index detector (RID) and a PL aquagel-OH MIXED column [12].

FT-IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer in the wavelength range of 4000–600 cm⁻¹ at a resolution of 4 cm⁻¹. The samples were ground to fine powder and mixed with potassium bromide (spectroscopic grade) to about 1% concentration.

Thermal stabilities of samples were performed using thermogravimetric analysis (TGA) on a simultaneous thermal analyzer (DTG-60, Shimadzu). The apparatus was continually flushed with nitrogen. The samples weighed between 8 and 10 mg and were run from room temperature to 600°C at a rate of 10°C/min.

2.3. Assay of DPPH- and ABTS-Scavenging Activity. 2-Diphenyl-β-picrylhydrazyl (DPPH) radical-scavenging activity (DRSA) was measured by the modified method described by Qiao et al. [13]. Briefly, 2 mL DPPH free radical (DPPH[•]) solution (400 μmol/L in ethanol) and 2 mL of sample solution (200 μg/mL, 400 μg/mL, 600 μg/mL, 800 μg/mL, and 1000 μg/mL in methanol) were added to a glass vial. The mixture was shaken and allowed to stand at 37°C in the dark for 30 min. Then, the absorbance of the mixture was measured at 517 nm against a blank. The scavenging percentage was calculated as the following equation:

$$\text{DRSA (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100, \quad (1)$$

where A₀ is the absorbance of the control system (DPPH[•] solution plus methanol with the absence of sample), A₁ is the absorbance of the sample system (DPPH[•] solution plus sample solution), and A₂ is the absorbance of the corresponding system (sample solution and ethanol with the absence of DPPH[•]). All measurements were performed in triplicate.

The antioxidant activity of samples was evaluated by the slightly modified 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method [14]. Firstly, a solution of 7 mM ABTS was prepared in 2.45 mM potassium persulfate and kept in dark at RT for 16 h, allowing the ABTS^{•+} formation, then, diluting the ABTS^{•+} solution with ethanol (1 : 80) to a certain concentration with an absorbance at 734 nm between 0.7 and 0.8. The antioxidant activities of the samples were determined as the following. 0.1 mL sample solution (200 μg/mL, 400 μg/mL, 600 μg/mL, 800 μg/mL, and 1000 μg/mL in 70% ethanol aqueous) and 3.9 mL ABTS^{•+} solution were added into a glass vial and incubated at room temperature in dark conditions for 10 min. The absorbance of the mixture was measured at 734 nm against a blank (ABTS^{•+} solution without sample). The scavenging percentage was calculated as the following equation:

$$\text{ABTS (\%)} = \frac{A_0 - A_1}{A_0} \times 100, \quad (2)$$

where A₀ is the absorbance of blank and A₁ is the absorbance of the sample system. All measurements were performed in triplicate.

3. Result and Discussion

3.1. Yield and Sugar Composition. Yields and chemical compositions of the RG, P-AHP, and P-LHW were shown in Figure 1. AHP and LHW treatments removed most of the nonsugar composition in the RG and yielded 78.0% and 75.1% polysaccharides, respectively. In RG, nonsugar composition accounted about 25% of the mass weight. Ash may be the main composition of the nonsugar materials. About 28% mass left at 600°C in thermal analysis might confirm this speculation (Figure 2).

Polysaccharides from peach gum consisted of arabinose (30.6%–38.8%) and galactose (27.5%–40.3%) as the main neutral sugar composition followed by xylan (7.9%–11.8%) and minor amount of glucose (0.2%–0.3%), mannose (1.3%–3.2%), and uronic acids (3.7%–6.1%). This phenomenon suggested that arabinogalactans were the main chain of the polysaccharides from peach gum. It was in agreement with the structures of gum exudate polysaccharides from the trunk and fruit of the *Prunus persica* reported by Simas et al. and Simas-Tosin et al. [2–4]. These reports also have confirmed the substituents of the polysaccharides, such as xylose, glucose, and uronic acids, by NMR and GC-MS analysis [2–4]. The composition of P-AHP and P-LHW is similar to that of RG with quantitative differences. The reduction of the xylose content from 11.8% in RG to 8.1% in P-AHP and

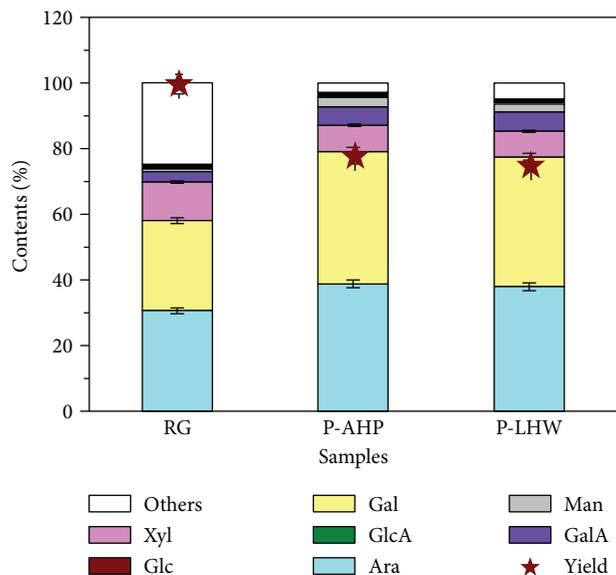


FIGURE 1: Yields and chemical composition of the polysaccharide fractions.

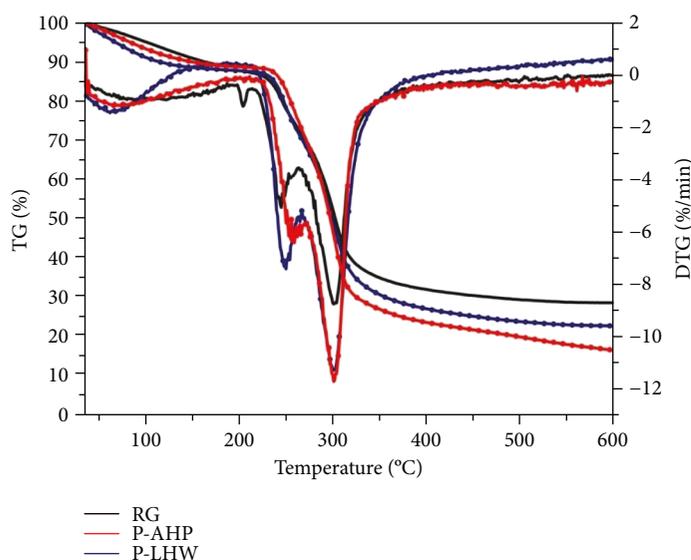


FIGURE 2: TG and DTG curves of the polysaccharide fractions.

7.9% in P-LHW, respectively, indicated the breaking of linkages between substituents and the main chain during AHP and LHW treatment. With the decrease of the xylose content, the relative contents of arabinose and galactose increased. However, the contents of glucose, mannose, and uronic acids were not affected by the treatments. This phenomenon might be ascribed to AHP and LHW which in favor the cleaving xylose rather than glucose, mannose, and uronic acid substituents due to a little more content of xylose than other minor amount sugars.

3.2. Molecular Weight and FT-IR Spectra. Molecular weight (weight average, \bar{M}_w and number average, \bar{M}_n) and polydispersities (PDI) are important parameters to evaluate the physicochemical properties and potential utility of

TABLE 1: Molecular weights (weight average, \bar{M}_w and number average, \bar{M}_n) and polydispersities (PDI) of the polysaccharide fractions.

	RG	P-AHP	P-LHW
\bar{M}_w	182500	78450	68420
\bar{M}_n	91860	67540	43580
PDI	2.0	1.2	1.6

polysaccharides [15]. Table 1 showed the molecular weights and the PDI of the polysaccharides. The polysaccharides in the RG had a higher \bar{M}_w (182500 g/mol) as compared to that of P-AHP (78450 g/mol) and P-LHW (68420 g/mol) fractions. This phenomenon indicated that AHP and LHW treatments introduced the degradation of polysaccharides, according with the results from yield and sugar analysis. The PDI of samples suggested that AHP and LHW treatments cleaved the linkages between substituents and backbone, enhancing the homogeneities of polysaccharides. It was consistent with the reduced xylose contents in these fractions shown in Figure 1.

IR spectroscopy is a widely used method for the determination of molecular structure and investigation of complex polymers. As shown in Figure 3, a broad peak at 3440 cm^{-1} was assigned to stretching vibration modes of O-H bound to carbons. A prominent C-H stretching band was observed at 2930 cm^{-1} . In the fingerprint region ($1000\text{--}1700\text{ cm}^{-1}$), many sharp and discrete absorption bands due to the various functional groups were observed. The absorbance peak at 1620 cm^{-1} was partial due to the intramolecularly absorbed water and the presence of carboxyl group. The peak at 1380 cm^{-1} was attributed to the symmetric deformation of $-\text{CH}_3$ and $-\text{CH}_2$ [16]. In addition, Marchessault and Liang reported that the bond around 1380 cm^{-1} also could be resulted from uronic acids [17]. The absorption band at 1040 cm^{-1} was attributed to the C-O-C stretching vibration. These data demonstrated that the peach gum was mainly composed of polysaccharides. The similarities of the IR spectra of three fractions might be ascribed to the similar composition of samples.

3.3. Thermal Analysis. Structural characteristics of polysaccharides influence the thermal behavior [18]. The TG (%) and DTG curves of the samples were shown in Figure 2. The weight loss peak at about 70°C with a rate of 1%/min corresponded to the evaporation of absorbed water. The decomposition of the polysaccharides was observed in the region at $200\text{--}350^\circ\text{C}$. The weight loss rate increased with increasing temperature and obtained the maximum at about 300°C with a rate of 8.9–11.7%/min. The DTG curves suggested that no obvious pyrolysis occurred at temperature higher than 350°C . As the temperature increased to 600°C , there were still about 28%, 17%, and 22% solid residues left for RG, P-AHP, and P-LHW, respectively. The shoulder mass loss peaks at about $240\text{--}250^\circ\text{C}$, at the weight loss rate of 5–8%/min, is probably assigned to the degradation of the branches, which were easily removed from the main chain and degraded to volatiles evolving out at relatively lower temperature, and

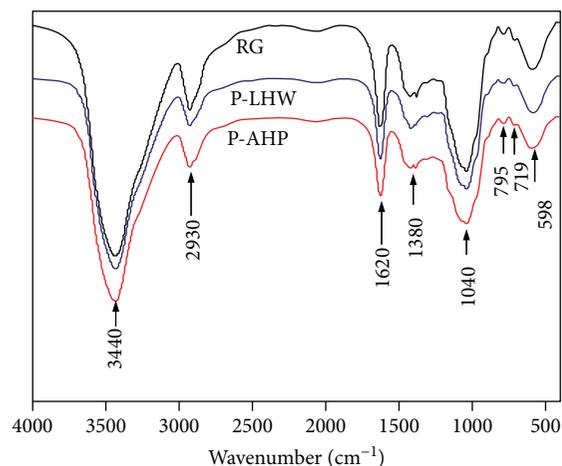


FIGURE 3: FTIR spectra of the polysaccharide fractions.

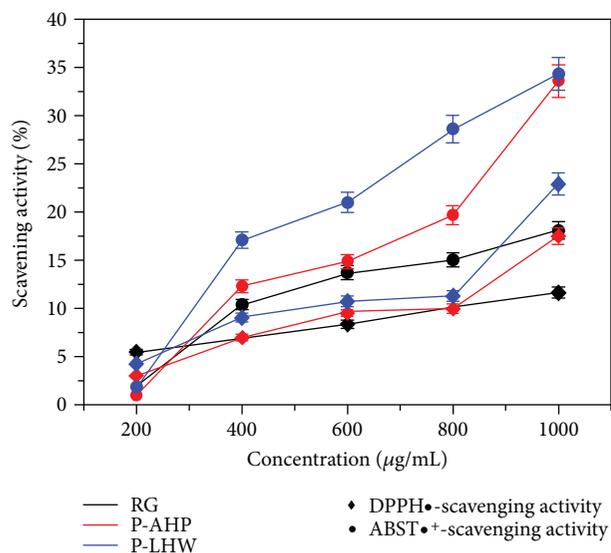


FIGURE 4: Activities of the polysaccharide fractions.

the backbone of the chain was pyrolyzed at much higher temperature [19]. As shown in Figure 2, the DTG curve of RG showed a low mass loss rate, suggesting a higher thermal stability of RG than P-AHP and P-LHW. It was mainly attributed to the higher molecular weight and ash ratio of RG as compared to treated samples (Table 1).

3.4. DPPH- and ABTS-Scavenging Activity. DPPH has been widely accepted as a tool for estimating the free radical-scavenging activities of antioxidants [13]. Yao et al. reported that the oligosaccharides derived from peach gum could scavenge 91.7% DPPH[•] at concentration of 100 mg/mL [20]. In this study, the activities of RG, P-AHP, and P-LHW were shown in Figure 4. The DPPH[•]-scavenging activities of fractions increased with the increase of concentration up to 1000 µg/mL. At a concentration of 1000 µg/mL, 11.6%, 17.5%, and 22.9% DPPH[•]-scavenging efficiencies were obtained for RG, P-AHP, and P-LHW, respectively. The favorable scavenging activity of treated samples may be due

to the higher content of the sugar composition as compared to RG. In addition, structural changes during treatments might also affect the activities of polysaccharide fractions. Taking P-AHP and P-LHW into comparison, P-LHW had a little low sugar content but a better scavenging activity than P-AHP. It is speculated that AHP and LHW treatments lead to different structure changes of polysaccharides, further lead to the difference in activity of polysaccharides.

ABTS assay has special chemical properties of formed free radicals and has been used to determine the antioxidant capacity of food products. The RG, P-AHP, and P-LHW scavenged 18.1%, 33.6%, and 34.3% ABTS^{•+} at concentration of 1000 µg/mL, respectively. However, due to the complexity of constituent and structure, the DPPH[•]- and ABTS^{•+}-scavenging mechanism of polysaccharides was not easily explained.

4. Conclusion

The polysaccharide in the peach gum was composed of arabinogalactans main chain substituted with xylose, glucose, mannose, and uronic acids. AHP and LHW treatments removed most of the nonsugar composition in the peach gum, introducing an increment in activity of polysaccharides. However, these treatments also led to the degradation of polysaccharides and the decrease of molecular weight and thermal stability of polysaccharides.

Data Availability

The data, in free formats, used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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