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Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities

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Abstract—A large number of polysaccharides are present in the pericarp tissues of harvested litchi fruits. A DEAE Sepharose fast-flow anion-exchange column and a Sephadex G-50 gel-permeation column were used to isolate and purify the major polysaccharides from litchi fruit pericarp tissues. Antioxidant activities of these major polysaccharide components were also evaluated. An aqueous extract of the polysaccharides from litchi fruit pericarp tissues was chromatographed on a DEAE anion-exchange column to yield two fractions. The largest amount of the polysaccharide fraction was subjected to further purification by gel filtration on Sephadex G-50. The purified product was a neutral polysaccharide, with a molecular weight of 14 kDa, comprised mainly of 65.6% mannose, 33.0% galactose and 1.4% arabinose. Analysis by Smith degradation indicated that there were 8.7% of (1→2)-glycosidic linkages, 83.3% of (1→3)-glycosidic linkages and 8.0% of (1→6)-glycosidic linkages in the polysaccharide. Furthermore, different polysaccharide fractions extracted and purified from litchi fruit pericarp tissues exhibited strong antioxidant activities. Among these fractions, the purified polysaccharide had the highest antioxidant activity and should be explored as a novel potential antioxidant.

Keywords: Litchi fruit; Polysaccharide; Purification; Composition; Infrared spectra; Antioxidant activity

1. Introduction

Polysaccharides are generally present in the cellular walls of fruits and vegetables,[1–3] and these can give a thickening, stabilizing or gelling effect to maintain fruit texture.[3] In addition, most polysaccharides that are used in the industry are from plant origins.[1,4,5]

Litchi (*Litchi chinensis* Sonn.) is a tropical to subtropical fruit with a delicious taste and lovely shape.[6–9] Litchi fruit pericarp tissues contain a large number of polysaccharides. Published data indicates that plant polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants.[10–12] To date, no investigation has been carried out on polysaccharides that may account for the textural properties and antioxidant activities of litchi fruit. Identification of the polysaccharides is necessary to better effectively exploit the structure and functional properties of these substances.

The objective of this study is to extract and purify the major polysaccharides of litchi fruit pericarp tissues using a DEAE Sepharose fast-flow anion-exchange column and a Sephadex G-50 column. The antioxidant activities of these major polysaccharides are also evaluated.

2. Materials and methods

2.1. Materials

Fresh fruits of litchi (*L. chinensis* Sonn.) cv. Huaizhi at the commercially mature stage were picked from a

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commercial orchard in Guangzhou, China. Fruits were selected for uniformity of shape and colour.

2.2. Chemicals

1,1-Diphenyl-2-picryldydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), dihydronicotineamidadenine dinucleotide (NADH), thiobarbituric acid (TBA), butylated hydroxyanisole (BHA), deoxyribose, L-arabinose, D-xylose, D-fructose, D-galactose and D-mannose were purchased from Sigma Chemical Co. (St Louis, MO, USA), while DEAE Sepharose (fast flow) and Sephadex G-50 were from the Pharmacia Co. (Sweden). All other chemicals used were of analytical grade.

2.3. Extraction of polysaccharides

Polysaccharide extraction was conducted by the method of Qin et al. with some modifications.[13] Litchi fruit pericarp tissues (5.0 g) were extracted three times with 100-mL portions of distilled water for 2 h at 30 °C and then filtered through Whatman No. 1 paper. The filtrates were combined and concentrated to 25 mL using a rotary evaporator at 30 °C. The proteins in the extract were removed using the Sevag reagent.[14] After removal of the Sevag reagent, 100 mL of anhyd EtOH was added to the extract, and the mixture was kept overnight at 4 °C to precipitate the polysaccharides.

2.4. Separation and purification of the polysaccharides

A DEAE Sepharose fast-flow column (16×100 mm) was used to isolate negatively charged polysaccharides from nonnegatively charged polysaccharides. The column was eluted with distilled water for 50 min, followed by 0.5 M NaCl for 50 min, at a flow rate of 0.3 mL/min. The major polysaccharide fractions were collected with a fraction collector and concentrated using a rotary evaporator at 65 °C. A 0.2-mL sample of the fraction with the largest amount of polysaccharide material was then loaded onto a Sephadex G-50 gel column (10×300 mm) and eluted with 25 mL of distilled water at a flow rate of 0.5 mL/min. The fraction with the highest polysaccharide content was collected and then freeze dried. All of these fractions were assayed for sugar content by the phenol–sulfuric acid method.[15]

2.5. Measurement of molecular weight of the purified polysaccharide

The molecular weight of the polysaccharide purified from litchi fruit pericarp tissues was determined by gel-permeation chromatography (GPC) by the method of Yamamoto et al.,[16] in combination with a high-performance liquid chromatography instrument (Waters 5215, USA) equipped with an Ultrahydrogel column, a model 410 refractive index detector and a Millennium 32 Workstation. The column was eluted with distilled water at a flow rate of 0.6 mL/min. Dextran standards with different molecular weights (4400, 9900, 21,400, 43,500, 124,000, 196,000, 277,000 and 845,000 Da) were used for the calibration curve.

2.6. Infrared spectral analysis of the polysaccharides

The IR spectrum of the polysaccharide purified from litchi fruit pericarp tissues was determined using a Fourier transform infrared spectrophotometer (FTIR, Bruker, Germany) equipped with OPUS 3.1 software. The purified polysaccharide was ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–500 cm^{-1}.[17]

2.7. Analyses of monosaccharide compositions and glycosidic linkage

The polysaccharide (10 mg) was hydrolyzed with 10 mL of 2 M trifluoroacetic acid (TFA) at 120 °C for 6 h.[18] Derivation was then carried out using the trimethylsilylation reagent according to the method of Guentas et al.[19] The trimethylsilylated derivatives were loaded onto a HP 5 capillary gas chromatography (GC) column equipped with a flame-ionization detector (FID), using inositol as the internal standard. The operation was performed using the following conditions: H_2: 16 mL/min; air: 150 mL/min; N_2: 20 mL/min; injection temperature: 230 °C; detector temperature: 230 °C; column temperature programmed from 130 to 180 °C at 5 °C/min, holding for 2 min at 180 °C, then increasing to 220 °C at 5 °C/min and finally holding for 3 min at 220 °C.

Analysis of the glycosidic linkages was conducted using Smith degradation procedures.[20] The polysaccharide (20 mg) was incubated with 40 mL of 15 mM sodium metaperiodate for 120 h at 4 °C. Excess sodium metaperiodate was destroyed by the addition of 50 μL of ethylene glycol. The product was reduced with 30 mg of $NaBH_4$, then dialyzed against distilled water, and finally hydrolyzed in 2 M TFA for 6 h at 120 °C. The sugars that were released were trimethylsilylated, and the resulting trimethylsilylated ether derivatives were analyzed by GC.

2.8. Assay for antioxidant activity

2.8.1. Evaluation of DPPH scavenging activity. The free-radical scavenging activity was measured by the method of Shimada et al. with some modifications.[21] Samples were dissolved in 10 mL of distilled water at 0 (control), 50 or 100 μg/mL. Then 2 mL of 0.2 mM DPPH in EtOH was added to 1 mL of the sample solution. The absorbance at 517 nm was measured after

20 min of incubation at 25 °C. In the study, BHA at 50 or 100 μg/mL was used as a positive control. The inhibition of DPPH radicals by the samples was calculated according to the following equation: DPPH-scavenging activity (%) = [1 − absorbance of sample/absorbance of control] × 100.

2.8.2. Evaluation of hydroxyl radical scavenging activity. The hydroxyl radical-scavenging activity was measured by the method of Ghiselli et al.[22] Samples were dissolved in 10 mL of distilled water at 0 (control), 0.5 or 1 mg/mL. The sample solution (0.1 mL) was mixed with 0.8 mL of reaction buffer [0.2 M phosphate buffer (pH 7.4), 1.75 mM deoxyribose, 0.1 mM ferrous ammonium sulfate and 0.1 mM EDTA] and 0.1 mL of 1.0 mM ascorbic acid, and 0.1 mL of 10 mM H_2O_2 was then added to the reaction solution. The reaction solution was incubated for 10 min at 37 °C and then 0.5 mL of 1% thiobarbituric acid and 1 mL of 2.8% trichloroacetic acid were added to the mixture. The mixture was boiled for 10 min and cooled on ice. The absorbance of the mixture was measured at 532 nm. Percent inhibition of deoxyribose degradation was calculated as (1 − absorbance of sample/absorbance of control) × 100. In the study, BHA at 0.5 or 1 mg/mL was used as a positive control.

2.8.3. Determination of superoxide anion-scavenging activity. The superoxide anion-scavenging activity was measured by the method of Robak and Gryglewski[23] with a minor modification. Samples were dissolved in 10 mL of distilled water at 0 (control), 50 or 100 μg/mL. A 1-mL aliquot of each sample solution was mixed with 1 mL of 0.1 M phosphate buffer (pH 7.4) containing 150 μM NBT, 60 μM PMS and 468 μM NADH. After 5 min of incubation at 25 °C, the absorbance was measured at 560 nm. The superoxide anion-scavenging activity was calculated as scavenging activity (%) = (1 − absorbance of sample/absorbance of control) × 100. BHA at 50 or 100 μg/mL was used as a positive control in the study.

2.9. Data handling

Data were expressed as means ± standard deviations of three replicated determinations. Originpro (Version 7.5, Origin Lab Inc.) statistical computer software was used for data analysis.

3. Results and discussion

3.1. Identification of polysaccharide components

An aqueous extract of polysaccharides from litchi fruit pericarp tissues was chromatographed on a DEAE anion-exchange column to yield two peaks, F1 and F2 (Fig. 1), with nonnegatively charged polysaccharide (F1) accounting for 82% of the total polysaccharides. The polysaccharide content of F1 was 99.5%. Thus, F1 was subjected to gel filtration on Sephadex G-50 and yielded a single fraction (F01), with a retention time of about 20 min (Fig. 2). F01 was collected for further identification of structure and monosaccharide compositions.

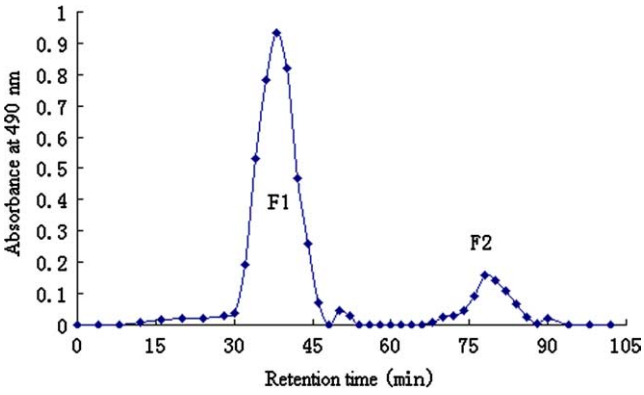

Figure 1. DEAE sepharose chromatogram of the crude polysaccharides extracted from pericarp tissues of litchi fruit.

The polysaccharide content of F01 was 99.9%. Monosaccharide compositions of the F01 fraction can be determined by the TFA hydrolysis method.[18,19] The F01 fraction consisted of 65.6% mannose, 33.0% galactose and 1.4% arabinose in terms of the molar proportions (Fig. 3).

Table 1 indicates the molar percentage of the purified polysaccharide based on Smith degradation products. There were 8.7% of the (1→2)-glycosidic linkages, 83.3% of the (1→3)-glycosidic linkages and 8.0% of the (1→6)-glycosidic linkages. However, the undetectable erythritol indicated that no (1→4)-glycosidic linkages were present in the purified polysaccharide.

The molecular weight of the polysaccharide was determined by gel-permeation chromatography.[16] The equa-

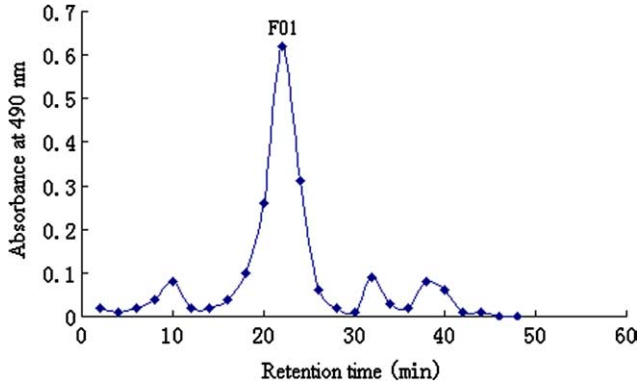

Figure 2. Sephadex G-50 gel chromatogram of the polysaccharides purified by a DEAE sepharose column.

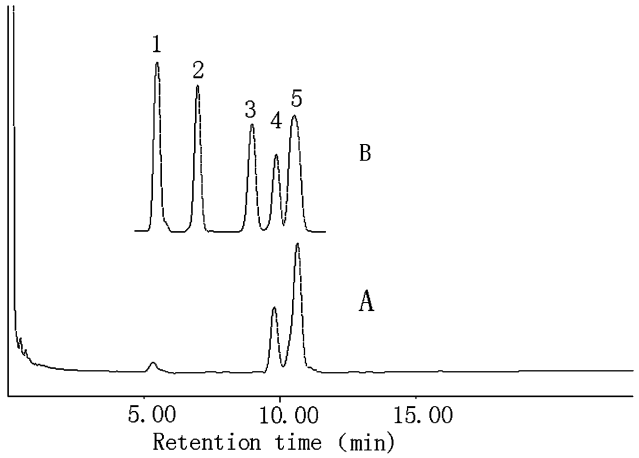

Figure 3. Gas chromatogram of monosaccharide compositions of the polysaccharide purified from pericarp tissues of litchi fruit: (A) F01 fraction; (B) (standard monosaccharide): (1) arabinose, (2) xylose, (3) fructose, (4) galactose and (5) mannose.

Table 1. Smith degradation of the polysaccharide (F01) purified from pericarp tissues of litchi fruit

	Molar percentage
Glycerol	16.7 ± 0.3
Erythritol	—[a]
Arabinose	—[a]
Mannose	53.5 ± 0.6
Galactose	29.8 ± 0.4

[a] Undetectable.

tion of the standard curve was drawn as $\log MW = 121.9 - 9.17t + 0.242t^2 - 0.00217t^3$ (where MW represents the molecular weight, while t represents elution time) using the Millennium 32 software. The molecular weight of the purified polysaccharide was estimated to be 14 kDa.

The infrared spectrum of the purified F01 fraction, as shown in Figure 4, displayed a broad stretching intense characteristic peak at around 3407 cm^{-1} for the hydroxyl group,[24] and a weak C–H stretching band at 2931 cm^{-1}. The peak around 2360 cm^{-1} also indicates

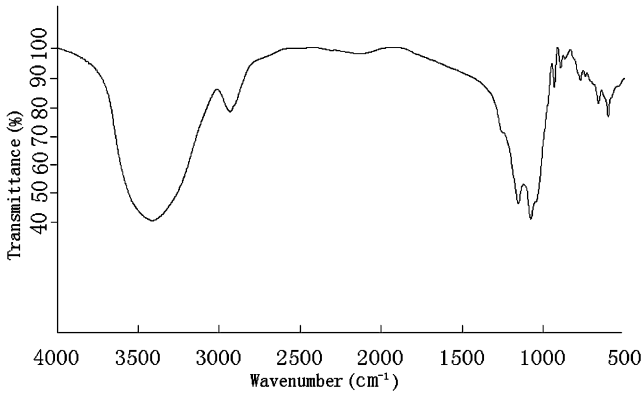

Figure 4. FTIR spectra of the polysaccharide purified from pericarp tissues of litchi fruit.

aliphatic C–H bonds. Two stretching peaks at 1077 and 1154 cm^{-1} suggest the presence of C–O bonds. The absorption of 932 cm^{-1} is due to antisymmetrical ring vibrations.[24] The peak at around 893 cm^{-1} is characteristic for β-D-mannose.[25] A band of absorption at 771 cm^{-1} represents symmetrical ring vibrations. The peak at 600 cm^{-1} is due to O–H out-of-plane vibrations.[26]

3.2. Antioxidant activity of different polysaccharide fractions

Some polysaccharides have exhibited strong antioxidant capability.[10,12] In this investigation, different polysaccharide fractions extracted and purified from litchi fruit pericarp tissues showed a dose-dependent, radical-scavenging activity (Table 2). The radical-scavenging activity enhanced with using increasing concentration. However, the radical-scavenging activity of these polysaccharides was lower than that of the BHA used in this study. Furthermore, the crude polysaccharides exhibited a relatively low level of radical-scavenging activity. After purification by means of a DEAE anion-exchange column, the nonnegatively charged fraction (F1) had a higher antioxidant activity than the crude polysaccharides. The polysaccharide fraction (F01) that was

Table 2. Radical-scavenging activities of the different polysaccharides extracted and purified from pericarp tissues of litchi fruit[a]

Sample	Amount (μg)	DPPH scavenging activity (%)	Hydroxyl radical scavenging activity (%)	Superoxide anion scavenging activity (%)
Crude polysaccharides	50	17.6 ± 0.3	21.4 ± 0.4	22.7 ± 0.5
	100	19.9 ± 0.4	25.6 ± 0.7	27.6 ± 0.6
F1 fraction	50	38.4 ± 1.1	42.7 ± 1.3	43.2 ± 1.1
	100	48.5 ± 1.4	47.9 ± 1.2	47.6 ± 1.2
F01 fraction	50	40.6 ± 0.9	46.8 ± 1.4	50.7 ± 1.6
	100	54.1 ± 1.5	53.1 ± 1.8	62.5 ± 2.0
BHA	50	68.3 ± 1.1	84.2 ± 1.4	78.9 ± 1.7
	100	76.6 ± 1.2	90.7 ± 1.7	85.7 ± 1.6

[a] Data are presented as mean ± standard deviations ($n = 3$). F1 fraction: polysaccharide purified partially by a DEAE Sepharose chromatography, and F01 fraction: polysaccharide purified further by a Sephadex G-50 gel-permeation chromatography.

purified further by the gel-filtration column exhibited the highest antioxidant activity. These results indicate that polysaccharides from litchi fruit pericarp tissues have strong antioxidant activities and should be explored as novel potential antioxidants.

4. Conclusions

A purified polysaccharide from litchi fruit pericarp tissues was obtained using DEAE Sepharose anion-exchange and gel-filtration chromatography. The purified polysaccharide was a neutrally charged species, with a molecular weight of 14 kDa, and comprised mainly of 65.6% mannose, 33.0% galactose and 1.4% arabinose. Analysis by Smith degradation indicated that there were 8.7% of (1→2)-glycosidic linkages, 83.3% of (1→3)-glycosidic linkages and 8.0% of (1→6)-glycosidic linkages in the polysaccharide. Different polysaccharide fractions extracted and purified from litchi fruit pericarp tissues exhibited strong antioxidant activities. Among them, the purified polysaccharide had the highest antioxidant activity and should be explored as a novel potential antioxidant.

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