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CHEMICAL COMPOSITIONS, A-GLUCOSIDASE AND A-AMYLASE INHIBITORY ACTIVITIES OF CRUDE POLYSACCHARIDES FROM THE ENDODERMIS OF SHADDOCK *(CITRUS MAXIMA)*

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Abstract - The chemical composition of shaddock mainly includes polyphenols, proteins and polysaccharides. However, polysaccharides from shaddock materials have received much less consideration than polyphenols (Fellers et al., 1990). Herein the chemical compositions, α-glucosidase and α-amylase inhibitory activities of crude polysaccharides from the endodermis of shaddock were investigated. The exopolysaccharides (EPS) exhibited a broad and intense peak at 3300-3400 cm^{-1} that characterized the absorption of the hydroxyl group, and one weak C-H band at around 2941.3 cm^{-1} in the IR spectrum. The content of neutral sugars in EPS was determined as 37.16%. The content of acidic sugar in EPS was determined as 33.71%. EPS exhibited the highest content of neutral sugar. The content of proteins in EPS was 5.75%. The content of polyphenols was 6.52%. The EPS mainly consisted of four types of polysaccharides with molecular weights of 110 kD, 68 kD, 31 kD and 12 kD. The crude EPS showed significantly higher inhibitory effects on α-glucosidase and α-amylase (inhibition to 74.12% and 86.59%, respectively).

Key words: Endodermis of shaddock, crude polysaccharides, chemical compositions,α-glucosidase and α-amylase inhibitory activities

INTRODUCTION

The grapefruit (*Citrus paradisi*), is a subtropical citrus tree known for its bitter fruit. It is an 18th-century hybrid first bred in Barbados (Carrington and Fraser, 2003). It has also been misidentified with the pomelo or shaddock (*C. maxima*), one of the parents of this hybrid. The grapefruit was known as the *shaddock* or *shattuck* until the 19th century. Its current name alludes to the clusters of the fruit on the tree, which often appear similar to grapes (Kumamoto et al., 1987)

The shaddock contains polyphenols, proteins, and polysaccharides (Sakamoto et al., 1996). Great advances have been made in chemical and bioactive studies of the polyphenols from the shaddock in recent decades. In the field of polyphenolic compounds, grapefruit contains flavanone, naringin, alongside the two furanocoumarins, bergamottin and dihydroxybergamottin Xiao et al., 2011a, b). It is via the inhibition of this enzyme that grapefruit increases the effects of a variety of drugs by increasing their bioavailability (Garg et al., 1998; Armando et al., 1997). However, polysaccharides from shaddock materials have received much less consideration than polyphenols (Fellers et al., 1990). Herein the chemical compositions, α-glucosidase and α-amylase inhibitory activities of crude polysaccharides from the endodermis of shaddock are presented.

MATERIALS AND METHODS

The shaddock was collected from Nanning, China. 2-Deoxy-D-ribose, nitrotetrazolium blue chloride

(NBT), phenazine methosulfate (PMS), linoleic acid, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Aladdin Reagent Int. (Shanghai, China). Nicotinamide adenine dinucleotide (NADH) and Ferrozine were obtained from Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). Ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA) and D-mannitol were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All other reagents and solvents were of analytical grade. All aqueous solutions were prepared by using newly double-distilled water.

The total content of polysaccharides was determined by the phenol-sulfuric acid method (Dubois et al., 1956). The concentration of proteins was measured according to Bradford's method (Bradford, 1976) and the content of total phenols was determined by the Folin-Ciocalteu method (Xu et al., 2010).]

Extraction of crude polysaccharides

The dry endodermis of shaddock were extracted with 10 times distilled water at 90°C for 2 h and repeated twice. The extracts were centrifuged to remove the contaminants. The supernatant was concentrated by the rotary evaporation method and precipitated with 95% alcohol. The precipitate was then dissolved with water and dialyzed to remove small molecules. The dialyzed solution was freeze-dried to yield a crude extracellular polysaccharide powder (EPS). The extraction processes are shown in Scheme 1.

Determination of the molecular weight of EPS

The molecular weight of EPS was determined by gel permeation chromatography. Samples were dissolved in 0.02 M phosphate buffer solution and centrifuged at 16 000 r/min for 10 min, and then passed through a 0.45 μm filter. 20 μl of the supernatant was injected into a Shodex SB-804 HQ GPC column (300×8 mm) with a Shodex SB-G guard column (50×6 mm) (obtained from Showa Denko K.K. (Tokyo, Japan)). The GPC system was maintained at 45 °C and eluted with phosphate buffer solution at a rate of 0.3 ml/min. The molecular weight was calculated by the calibration curve obtained by the standard dextrans (T3, T6, T10, T40, T100, T500, and T1000).

Analysis of monosaccharide composition

The EPS was hydrolyzed in 4 mol/L trifluoroacetic acid (TFA) for 6 h at 120°C in a sealed glass tube. The residual acid was removed under vacuum and then the hydrolyzates and monosaccharides were analyzed on a Dionex ICS2500 system with a CarboPac PA20 column (150 mm × 3 mm i.d.) and an ED50A detector. The temperature of column was kept at 30°C and 25 μL aliquot was injected for each run. The elution was realized by a gradient system (Table 1) with H_2O, 250 mmol/L NaOH and 1 mol/LNaAc.

Table 1 The gradient elution system for IC.

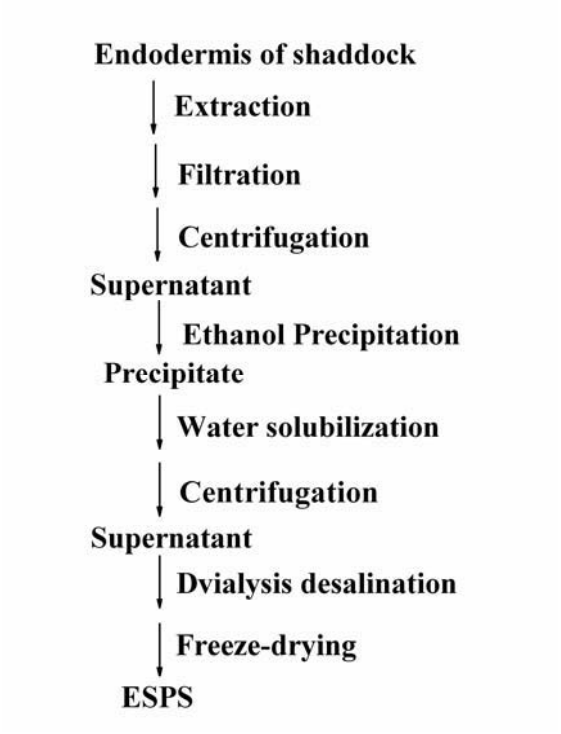
Time (min)	H_2O (%)	250 mmol/L NaOH (%)	1 mol/L NaAc (%)
0.0	99.2	0.8	0
30.0	99.2	0.8	0
30.1	94.2	0.8	5
40.0	79.2	0.8	20
40.1	20	80	0
60.0	20	80	0

IR spectroscopy

IR spectroscopy was used to investigate the vibrations of molecules and polar bonds between the different atoms. Structures of polysaccharides, such as monosaccharide types, glucosidic bonds and functional groups, could be analyzed using IR spectroscopy. The IR spectrum of EPS was recorded with a Nicolet 5700 IR spectrometer with the range of 4000-400 cm^{-1}.

Determination of α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of crude EPS was determined according to the chromogenic method described by Tremblay et al. with slight modifications (Ryu et al., 2010). The substrate solu-



Scheme 1. Scheme of EPS extraction.

tion *p*-nitrophenyl α-D-glucopyranoside (pNPG) was prepared with 0.1 M Na-phosphate buffer (pH 6.8). The reaction mixture was as follows: 0.1 mol/L Na-phosphate buffer (pH 6.8), 2 mL; 5 mg/mL EPS solution, 20 μL; 1 mg/mL reduced glutathione, 50 μl; 1U/μL α-glucosidase, 20 μL. The mixed solution was incubated at 37.5°C for 10 min. The enzymatic reaction was initiated by adding saturated pNPG and the reaction mixture was incubated for another 30 min at 37.5°C. The catalytic reaction was terminated by addition of 10 mL of 0.1 M Na_2CO_3 solution. The reaction system without polysaccharides was used as a blank test and the system without α-glucosidase was used as a background test. The Na-phosphate buffer (pH 6.8) was used as the zero-setting solution for the determination of the absorbance at the wavelength of 400 nm. The inhibitory rate of sample on α-glucosidase was calculated by the following formula.

$$\text{Inhibition percentage (\%)} = (A_{blank}-(A_{sample}-A_{background})) \times 100/A_{blank}$$

α-Amylase inhibitory activity

The α-amylase inhibitory activity of crude EPS was determined according to the method described by Kim et al. (2000) with slight modifications. Starch azure (10 mg, Sigma Chemical Co.) which was used as a substrate, was suspended in 1.0 mL of a 0.05 mol/L Tris-HCl buffer (pH 6.9) containing 0.01 mol/L $CaCl_2$ and heated for 5 min at 92°C. The starch solution was then pre-incubated at 37°C for 5 min. The sample was dissolved in ultra pure water (the final concentration was 1.0 mg/mL) and 0.5 mL of PPA solution (2.8 U/mL) (Sigma Chemical Co.) in the above buffer was applied for each assay. The reaction was carried out at 37°C for 10 min and stopped by adding 0.5 mL of 50% acetic acid. The reaction mixture was then centrifuged at 4500 rpm for 5 min. The absorbance of the resulting supernatant at 595 nm was recorded. Acarbose was used as a positive control group. The α-amylase inhibitory activity was calculated as follows:

$$\text{PPA inhibitory (\%)} = ((A_c^+ - A_c^-) - (A_s - A_b))/(A_c^+ - A_c^-) \times 100$$

where A_c^+, A_c^-, A_s and A_b are defined as the absorbance of 100% enzyme activity (only the solvent with the enzyme), 0% enzyme activity (only the solvent without the enzyme), a test sample (with the enzyme), and a blank (a test sample without the enzyme), respectively.

RESULTS AND DISCUSSION

Spectroscopic characteristics

The IR spectra of crude EPS are shown in Fig. 1. The EPS exhibited a broad and intense peak at 3300-3400 cm^{-1}, which characterized the absorption of a hydroxyl group, as well as one weak C-H band at around 2941.3 cm^{-1}. The relatively weak absorption peak at 1641.27 cm^{-1}, which was the characteristic IR absorption of protein, and some weak ones from 1450 to 1200 cm^{-1}, were also characteristic IR absorptions of polysaccharides. The IR absorptions at 1108.03 and 1021.15 cm^{-1} is the characteristic absorption

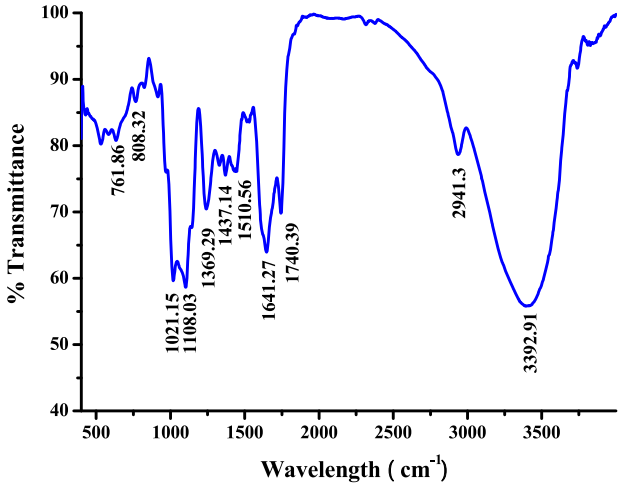


Fig. 1. IR spectra of crude EPS.

bands of pyranglycosides. The absorption peak at around 1740.39 cm^{-1} and a weak one at nearly 1369.3 cm^{-1} are indicative of the presence of carboxyl groups and carbonyl groups that pointed to IR absorption of uronic acids. These observations further confirmed that these extracts were composed of polysaccharide, protein and uronic acids.

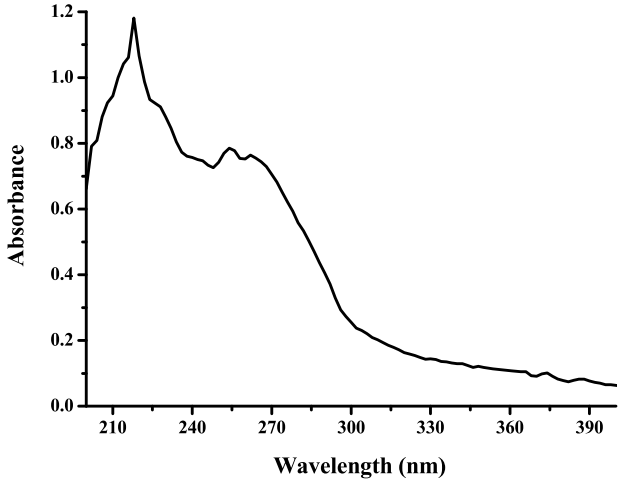


Fig. 2. UV absorbance spectra of crude EPS.

Figure 2 shows the UV absorbance spectra of crude polysaccharides. As seen from these data, the absorbance band of crude EPS is concentrated from 200-300 nm.

Chemical composition of crude EPS

As shown in Table 2**,** the EPS showed a similar neutral sugar content. The content of neutral sugar in EPS was 37.16%. The content of acidic sugars in EPS was 33.71%. EPS exhibited the highest content of neutral sugar. The content of proteins in the EPS was 5.75%. The content of polyphenols was 6.52%. The EPS was mainly composed of rhamnose, arabinose, galactose, glucose, xylose, mannose, ribose, galacturonic acid, and galacturonic acid with the following mole ratios 1.0 : 0.8 : 1.6 : 0.9 : 0.7 : 2.7 : 0.9 : 0.4 : 1.3. No fucose or fructose was found in the EPS.

Distribution of the molecular weights of crude EPS

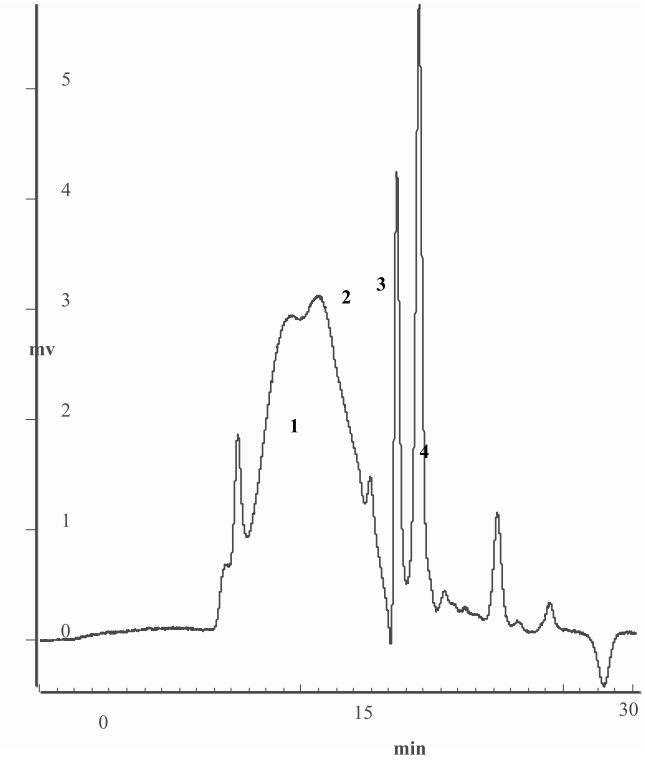


Fig. 3. Distribution of molecular weight in EPS.

Figure 3 shows the distribution of the molecular weight of the crude EPS. The EPS mainly consisted of four kinds of polysaccharides with molecular weights of 110 kD, 68 kD, 31 kD and 12 kD.

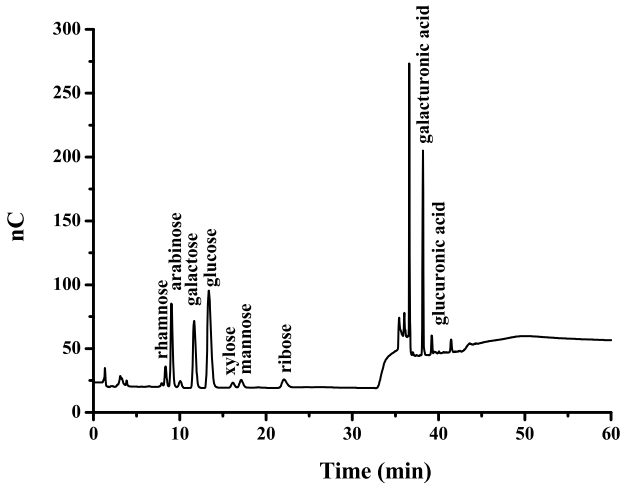


Fig. 4. IC chromatographs of monosaccharide composition in EPS.

Monosaccharide composition

The IC chromatograph of crude EPS is shown in Fig. 4. As seen in Fig. 4, the EPS was mainly composed of rhamnose, arabinose, galactose, glucose, xylose, mannose, ribose, galacturonic acid, and galacturonic acid with mole ratios of 1.0 : 0.8 : 1.6 : 0.9 : 0.7 : 2.7 : 0.9 : 0.4 : 1.3. No fucose or fructose was found in the crude polysaccharides.

α-Glucosidase and α-amylase inhibitory activities

Diabetes is characterized by high concentrations of blood sugar, which can cause serious complications in the kidneys, eyes and cardiovascular system. The treatment of diabetes therefore mainly focuses on reducing fluctuations in blood sugar and subsequent complications. The α-glucosidase and α-amylase inhibitors are currently used for diabetic treatment as oral hypoglycemic agents. The α-glucosidase inhibitors inhibit the degradation of disaccharides to monosaccharide. The α-glucosidase inhibitors are mostly evaluated by the determination of α-glucosidase inhibitory activity using pNPG as the reaction substrate.

The crude EPS showed significantly higher inhibitory effects on α-glucosidase and α-amylase to 74.12% and 86.59% activities, respectively. Recently, Chen et al. compared the glycosidase inhibitory effects of three polysaccharide-rich fractions from green tea, oolong tea, and black tea and found that the glycosidase inhibitory property appeared to be related to differences in the monosaccharide composition and molecular weight distribution of the polysaccharide. Tea leaves can be a resource of tea polysaccharides.

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