International Journal of Biological Macromolecules 91 (2016) 23–30

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Physicochemical properties and antioxidant activities of polysaccharides sequentially extracted from peony seed dreg

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ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 18 May 2016
Accepted 22 May 2016
Available online 24 May 2016

Keywords:
Polysaccharides
Peony seed dreg
Sequential extraction
Physicochemical properties
Antioxidant activities

ABSTRACT

The sequential extraction of peony seed dreg polysaccharides (PSDP) with hot buffer (HBSS), chelating agent (CHSS), dilute alkaline (DASS) and concentrated alkaline (CASS) yielded four different polysaccharide fractions. Based on their absorptions at 3600–3200 cm^{-1} and 1200–800 cm^{-1}, these fractions were confirmed to be polysaccharides. The properties of four PSDPs displayed some slight differences. The CASS showed the highest peak temperature and endothermic enthalpy. The emulsifying activity and emulsifying stability of four PSDPs exhibited a dose-dependent pattern; HBSS showed the highest emulsifying activity, and CHSS displayed the longest emulsifying stability. The four PSDPs also exhibited wide variations in their antioxidant activities. For example, i) CASS showed the highest DPPH radical scavenging activity, reducing power and ABTS radical scavenging activity; ii) HBSS exhibited the highest hydroxyl radical scavenging activity, and iii) CHSS displayed the higher ferrous ions chelating ability than others.

1. Introduction

While recorded cultivation of tree peony (*Paeonia suffruticosa Andr.*) in China dates back to the early part of the sixth century, it is widely cultivated in the other parts of the world including Japan, Korea, New Zealand, Europe, and North America [1]. Peonies, in addition to their ornamental values, are also used in Chinese medicine [2]. For example, paeoniflorin and paeonol, two bioactive components of tree peony, are known to exhibit analgesic and hypnotic effects [3] as well as to ameliorate progression of Spinocerebellar ataxia and Huntington's disease [4].

Plant polysaccharides from different sources have long been studied and widely used for a variety of purposes including food, animal feed, medicine, and papermaking [5]. Polysaccharides also exhibit an array of biological activities that include antioxidant, immunomodulatory, antitumor, gastrointestinal protection, antidiabetic, and hepatoprotective effects [6].

Consequent to changes in methods of extraction and processing, these polysaccharides tend to acquire a wide diversity in their structure and function [7]. For example, when okra cell polysaccharides were sequentially extracted using hot buffer (HBSS), chelating agent (CHSS), dilute alkaline (DASS) and concentrated alkaline (CASS), these extracts yielded products with pronounced differences in their composition, sugar linkage, and rheological properties [8]. To the best of our knowledge, there is no such report about the extraction, physicochemical properties and function of polysaccharides from peony.

Peony seed dreg, a by-product of oil processing, is almost exclusively used as animal fodder and fertilizer. However, the potential use of constituents of peony seed dreg for functional food remains unexplored. Therefore, we have sequentially extracted peony seed dreg with hot buffer (HBSS), chelating agent (CHSS), dilute alkaline (DASS) and concentrated alkaline (CASS) and examined their physicochemical and antioxidant properties. The obtained results of thermal, emulsifying and antioxidant properties of PSDPs are important for peony seed dreg in potential industrial applications of functional foods.

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http://dx.doi.org/10.1016/j.ijbiomac.2016.05.082
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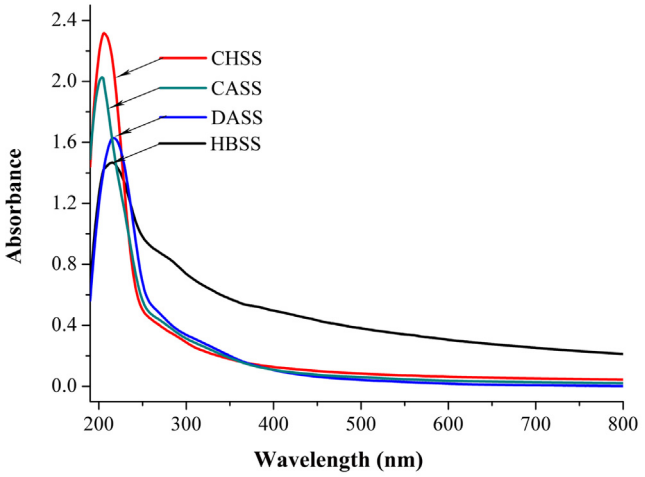

Fig. 1. Scan of peony seed dreg polysaccharides within the wavelength range of 190–800 nm.

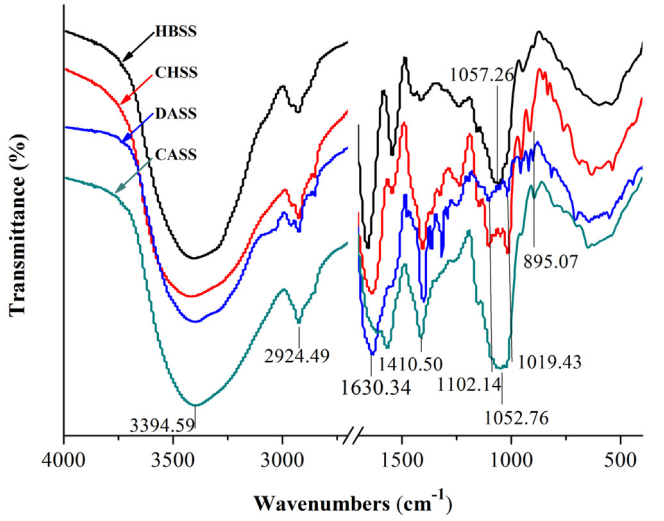

Fig. 2. FT-IR spectrometric analysis of the peony seed dreg polysaccharides within the frequency range of 4000–400 cm^{-1}.

2. Materials and methods

2.1. Materials

The peony seed dreg, the residue remaining after the oil extraction with screw press expression, was obtained from Anhui Tongling Ruipu Peony Industry Development Co., Ltd. The dregs were dried at 60 °C to a stable moisture content of less than 4%. Dry samples were ground to a fine powder and the samples were stored at 4 °C. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI (Shanghai) Development Co., Ltd. Ferrozine and 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) were obtained from Aladdin Reagent Co., Ltd., China. All other chemicals used for analysis were analytical grade.

2.2. Isolation of alcohol-insoluble solid

The peony seed dreg was homogenized twice with 70% (v/v) aqueous ethanol at room temperature. After filtration, the insoluble residues were combined and washed with two volumes of chloroform/methanol (1/1, v/v) with gentle stirring for 30 min to remove low molecular weight (colored) compounds [8]. After filtration, the solids were washed with acetone and air-dried (alcohol-insoluble solids, AIS).

2.3. Sequential extraction of peony seed dreg AIS

Further extractions of AIS fraction was performed as described by Sengkhamparn et al. [8]. Briefly, peony seed dreg AIS (20 g) was sequentially extracted with a) 0.05 M sodium acetate buffer at pH 5.2 and 70 °C for 30 min (hot buffer soluble solids, HBSS), b) 0.05 M EDTA and 0.05 M ammonium oxalate in 0.05 M sodium acetate buffer at pH 5.2 and 70 °C for 30 min (chelating agent soluble solids, CHSS), c) 0.05 M sodium hydroxide and 20 mM $NaBH_4$ at 4 °C for 30 min (diluted alkali soluble solids, DASS), and d) 6 M sodium hydroxide and 20 mM $NaBH_4$ at 4 °C for 2 h (concentrated alkali soluble solids, CASS). This extraction sequence was repeated until the total sugar content of the last supernatant was lower than 40 μg/mL. After each extraction, the soluble was separated from the insoluble residue by centrifugation (10000 rpm for 25 min). The supernatants were dialyzed in tubing with a pore size equivalent to a nominal molecular weight cut-off (MWCO) of 8000 against distilled water at room temperature for 48 h. After dialysis, the remaining products were freeze-dried and stored until further analysis.

2.4. UV absorption peak detection

The peony seed dreg polysaccharides were dissolved in distilled water to a final concentration of 0.1%. The UV absorption spectra of the samples were recorded in the wavelength range of 190–800 nm [9].

2.5. FT-IR spectrometric analysis

Fourier transform infrared (FT-IR) spectroscopy was performed to obtain the compositional information of PSDPs. The PSDPs were incorporated into a KBr powder and then pressed into a 1.0-mm-thick pellet. FT-IR measurement was obtained over the frequency range of 4000–400 cm^{-1} using a Thermo Nicolet 67 FT-IR spectrometer equipped with a DTGS detector with a resolution of 0.09 cm^{-1} [10].

2.6. Thermal characteristic analysis

The thermal properties of PSDPs were investigated by differential scanning calorimetry (DSC) [11]. A 3.8 mg sample was placed in the aluminum pot and then sealed with an empty pot as a reference. The thermal data were obtained at 10 °C min^{-1} in the temperature range of 20–225 °C under nitrogen atmosphere (at 50 cm^3 min^{-1}). The system was previously calibrated with indium (In) standard for temperature and enthalpy.

2.7. Determination of emulsifying properties

The emulsifying activity (EA) and emulsifying stability (ESI) of the PSDPs were determined by turbidity analyses [12]. A 3 mL aliquot of various concentrations of sample solution was mixed with 3 mL soybean oil and then homogenized for 1 min. A 100 μL of the homogenized sample was mixed with 10 mL 0.1% of sodium dodecyl sulphate (SDS) solution and absorbance was measured at 500 nm immediately (A_0) and 10 min later (A_{10}) (0.1% SDS was used as control). A_0 was used as a reference for emulsifying activity (EA) [13]. The emulsifying stability (ESI) was calculated as follow [14]:

$$ESI = \frac{A_0 \times \Delta T}{A_0 - A_{10}}$$