International Journal of Biological Macromolecules 95 (2017) 809–817

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Characterization, antioxidant activity and immunomodulatory activity of polysaccharides from the swollen culms of *Zizania latifolia*

Mingchun Wang[a,b,c,*], Peilei Zhu[a,c,d], Shiwei Zhao[a,b], Chenzhipeng Nie[a,b], Naifu Wang[a,b], Xianfeng Du[a,b], Yibin Zhou[a,b,*]

[a] Department of Food Science and Engineering, Anhui Agricultural University, Hefei, 230036, China
[b] Anhui Province Agricultural Products Processing Engineering Laboratory, Hefei, 230036, China
[c] Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ, 08901, USA
[d] Institute of Horticulture, Anhui Academy of Agricultural Sciences, Hefei, 230031, China

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 2 December 2016
Accepted 3 December 2016
Available online 6 December 2016

Keywords:
Polysaccharide
Zizania latifolia
Characterization
Antioxidant activity
Immunomodulatory activity

ABSTRACT

The swollen culms of *Zizania latifolia* have been used as a vegetable and traditional herbal medicine in China, Japan, Korea and Southeast Asia countries. Up to date, there is little information about the polysaccharides from the swollen culms of *Zizania latifolia* and their potential bioactivities. In the present study, water extractable polysaccharide (ZLPs-W) and alkali extractable polysaccharide (ZLPs-A) was sequentially prepared from the swollen culms of *Zizania latifolia*. Both of ZLPs-W and ZLPs-A was found to be non-starch polydisperse heterpolysaccharide with β-type glycosidic linkage. ZLPs-W with triple helix conformation mainly composed of GalA, Glc and Gal. ZLPs-A without triple helix conformation mainly composed of Glc, Gal, Xyl and Ara. In *in vitro* antioxidant assay, ZLPs-W and ZLPs-A exhibited good scavenging activities. The EC50 of DPPH radical, superoxide radical and hydroxy radical scavenging activities for ZLPs-A is 1.87, 1.13 and 0.38 mg/mL compared with that for ZLPs-W is 2.95, 3.99 and 0.5 mg/mL, respectively. Moreover, *in vitro* cell assay revealed that ZLPs-W without cytotoxicity has higher immunomodulatory activity than ZLPs-A in terms of stimulation of phagocytic ability and NO production in murine macrophage RAW 264.7. At the treated concentration of 400 μg/mL and 100 μg/mL, ZLPs-W induced a highest phagocytosis index (1.76) and NO product (29.12 μmol/L), respectively. The results suggest that polysaccharide from the swollen culms of *Zizania latifolia* could be explored as potential natural antioxidant and immunomodulatory agents in medicine or functional food fields.

1. Introduction

Polysaccharides and oligosaccharides, consisting of a number of monosacccharides joined by glycosidic linkages in linear or branched chains, are usually considered as one class of active compounds in organisms [1–3]. Nowadays, natural polysaccharides derived from higher plant, animal, fungi, seaweed, and so forth, are demonstrated to have wide-ranging bioactivities, including antitumor, immunomodulatory, anti-inflammatory and antioxidant activity [4–7]. Thereinto, antioxidant and immunomodulatory activity is widely studied and is considered as two kinds of primary bioactivities. Specifically, arabinogalactan, galactomannan and pectic polysaccharides derived from higher plant, β-glucans and glycoproteins derived from mushroom, sulfated polysaccharides derived from seaweed have been all proved to possess potent antioxidant and immunomodulatory activity [1,4,8–10]. Owing to their safety and nontoxic properties, some of these polysaccharides have been successfully applied as antioxidant and immunomodulator [1,4,9]. Consequently, in view of their potential application in functional food and medical areas, there is growing interest in further pursuing isolation, characterization and bioactivity of diverse polysaccharides from natural products.

Zizania latifolia is a perennial aquatic vegetable widely cultivated in the most of regions of China and scattered locations throughout Japan, Korea and southeast Asia countries [11,12]. The largest cultivation area of this plant is located in the regions surrounding Tai Lake, including Jiangsu and Zhejiang province of China [11]. The swollen culms of *Zizania latifolia*, commonly known as *jiaobai* or *gaogua* in China, containing high nutrition, having a delicious taste, have been considered to be delicacy and one of the

* Corresponding authors at: Department of Food Science and Engineering, Anhui Agricultural University, Hefei, 230036, China.
E-mail addresses: wmc@ahau.edu.cn, tcwmc1107@gmail.com (M. Wang), zhouyibin@ahau.edu.cn (Y. Zhou).

http://dx.doi.org/10.1016/j.ijbiomac.2016.12.010
0141-8130/

three most famous vegetables in the areas south of the Yangtze River. According to the ancient Chinese medicine literature, swollen culms have long been used as traditional herbal medicine possessing the effects of heat-clearing, detoxifying, quenching thirst, diuresis and so on [13]. Due to its values, cultivation area of *Zizania latifolia* has been expanded so that swollen culms are largely harvested every year [11]. However, the fresh swollen culms and its fresh-cut products have a faster rate of quality deterioration during past-harvest period [14]. Therefore, it is important to seek effective storage and processing method. Extraction and utilization of bioactivity components is an effective method to digest overstocking of fresh swollen culms and thus promote its utilization efficiency. Polysaccharide is one of the main components, which extensively existed in plant tissues. To date, little information is available about the polysaccharides from swollen culms of *Zizania latifolia* and their potential bioactivity. Therefore, in the present study, water and alkali extractable polysaccharides sequentially extracted from swollen culms of *Zizania latifolia* were characterised firstly. Then, the potential antioxidant activities of polysaccharides were evaluated by *in vitro* assays of DPPH radical, superoxide radical and hydroxyl radical scavenging activity. Finally, immunomodulatory activities of polysaccharides were evaluated by *in vitro* cell assays using murine Macrophage RAW264.7.

2. Materials and methods

2.1. Materials and chemicals

The fresh swollen culms of *Zizania latifolia* were harvested in September of 2014 in Yuexi City, Anhui Province, China. They were sliced, dried, pulverized into powder, and kept at 4 °C until use. Dialysis tube (molecular weight cutoff, 5000 Da) was purchased from Shanghai Green Bird Science and Technology Development Co., Ltd. (Shanghai, China). Arabinose (Ara), galactose (Gal), galacturonic acid (GalA), glucose (Glc), glucuronic acid (GlcA), mannose (Man), rhamnose (Rha), xylose (Xyl), 1-phenyl-3-methyl-5-pyrazolonde (PMP) and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were analytical grade or chromatographic grade.

2.2. Preparation of polysaccharides from swollen culms of Zizania latifolia

The water extractable polysaccharides were prepared as the following method. Briefly, the powder of swollen culms was refluxed twice with 75% ethanol for 1.5 h at 70 °C. The resulting residues were dried in air and then extracted with hot distilled water (1:20, w/v) for 3.0 h at 90 °C for three times. The extracted solutions were combined, concentrated by rotary evaporator to 1/8 of the original volume. Then the polysaccharides were precipitated by adding with three volumes of anhydrous ethanol and kept at 4 °C for 24 h. The sediments from centrifugation were dissolved in distilled water and concentrated by rotary evaporator for a proper time to remove resident ethanol. The polysaccharides solutions were dialyzed, concentrated and freeze-dried to afford water extractable polysaccharides of the swollen culms of *Zizania latifolia* (ZLPs-W).

The water unextractable solid residues were then extracted with 0.5 M NaOH and 0.05 M $NaBH_4$ solution at 85 °C for 3 h for three times. The extracted solution was neutralized to pH 7 by hydrochloric acid and concentrated by rotary evaporator to 1/8 of the original volume. According to the above mentioned preparation method of ZLPs-W, the following steps in precipitating, dialyzing and freeze-drying were conducted to get the alkali extractable polysaccharides of the swollen culms of *Zizania latifolia* (ZLPs-A).

2.3. Preliminary characterization of ZLPs-W and ZLPs-A

2.3.1. Determination of contents of carbohydrate and protein

The carbohydrate content was determined by the phenol-sulfuric acid method using glucose as a standard [15]. The protein content was measured by the Bradford assay using bovine serum albumin as a standard [16].

2.3.2. FT-IR and UV spectroscopy analysis

FT-IR spectrum of ZLPs-W and ZLPs-A were obtained by using a Nicolet iS50 FT-IR Spectrometer (Thermo Scientific, USA) in the wavenumber range of 4000–500 cm^{-1}. The UV spectrum of ZLPs-W and ZLPs-A were recorded using Lambda 35 spectrophotometer (PerkinElmer Inc., USA) in the range of 200–400 nm.

2.3.3. Monosaccharide composition analysis

The monosaccharide composition of ZLPs-W and ZLPs-A was analyzed by HPLC according to the reported method [17]. Briefly, 5 mg of samples was hydrolyzed with 2 mol/L trifluoroacetic acid (2 mL) at 120 °C for 2 h. After derivatized with PMP, the PMP-labeled sugars were analyzed on a Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 μm, Agilent Technologies, USA) connected to an Agilent 1100 HPLC system (Agilent Technologies, USA). The wavelength of detection was 245 nm. Mobile phase was phosphate buffered saline buffer (PBS, 0.1 mol/L, pH 6.7) containing 17% acetonitrile at a flow rate of 1.0 mL/min, and injection volume was 20 μL.

2.3.4. Determination of molecular weight distribution

The molecular weight distribution was determined by high performance gel-permeation charomatography (HPGPC) using an Waters 1525 HPLC system equipped with a refractive index detector and a TSK-GEL G4000PW_{xl} column (7.8 × 300 mm, Tosoh Corp., Japan). The column oven temperature was set at 35 °C and the column was eluted with 0.1 mol/L Na_2SO_4 solution in PBS buffer (0.01 mol/L, pH 6.8) at a flow rate of 0.6 mL/min. T-series Dextran as standards were used to calculate molecular weight.

2.3.5. Congo red test

The conformation structure of ZLPs-W and ZLPs-A was established by helix-coil transition analysis according to Ogawa procedure with some modifications [18]. Briefly, 5 mg polysaccharide samples were mixed with 2 mL deionized water and 2 mL Congo red solution (80 μmol/L). NaOH solution (1 mol/L) was added to the mixture gradually to make the final concentration of NaOH to 0, 0.1, 0.2, 0.3, 0.4 and 0.5 μmol/L, respectively. Maximum absorption wavelength was measured using Lambda 35 spectrophotometer in the range of 200 nm to 800 nm after keeping the samples at room temperature for 5 min. The mixtures without polysaccharide samples were recorded as control.

2.4. Assays for antioxidant activity

2.4.1. Assay of DPPH radical scavenging activity

DPPH radical scavenging activity was determined by using the method of Wu et al. [5] with slight modifications. Briefly, 2.0 mL of 0.1–4 mg/mL sample solutions and 2.0 mL of 0.1 mmol/L DPPH solutions (dissolved in ethanol) were mixed and shaken vigorously. After incubated in the dark at room temperature for 30 min, the absorbance of reaction mixture at 517 nm was measured against a blank (50% ethanol solution). Vc was used as a positive control. DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity}(\%) = [A_0 - (A_1 - A_2)]/A_0 \times 100$$