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Simultaneous determination of molecular weights and contents of water-soluble polysaccharides and their fractions from *Lycium barbarum* collected in China

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ABSTRACT

Molecular weights and contents of water-soluble polysaccharides and their fractions in fifty batches of fruits of *Lycium barbarum* (wolfberry) collected from different regions of China, including Qinghai, Ningxia, Inner Mongolia, Xinjiang, and Gansu, were simultaneously determined using high performance size exclusion chromatography (HPSEC) coupled with multi angle laser light scattering (MALLS) and refractive index detector (RID) with the refractive index increment (*dn/dc*). Results showed that HPSEC chromatograms and molecular weight distributions of polysaccharides in *L. barbarum* collected from different regions of China were similar. Furthermore, the average contents of each polysaccharide fraction (peaks 1, 2, and 3) in crude polysaccharides of *L. barbarum* collected from Ningxia were similar with those of Inner Mongolia, Xinjiang, and Gansu, respectively. However, significant difference was found between polysaccharides in *L. barbarum* collected from Ningxia and Qinghai. Moreover, the average amounts of total polysaccharide fractions (peaks 1, 2, and 3) in the raw material of *L. barbarum* collected from Ningxia were significantly higher than that of Qinghai. These results may contribute to the rational usage of *L. barbarum* produced in China, and are beneficial for the improvement of their quality control. Results suggested that HPSEC-MALLS-RID with the *dn/dc* method could be used as a routine method for the quality evaluation of polysaccharides from natural resources and their products.

1. Introduction

Lycium barbarum L., known as wolfberry, is a Solanaceous defoliated shrubbery, which is a well-known medicine and food dual purposes plants used in China [1]. The fruit of *L. barbarum* has been widely used for preventing and treating various diseases in East Asia for more than 2000 years [2]. Due to their various benefits of strengthening the kidneys, nourishing the liver, moistening the lungs and improving eyesight, numerous wolfberry products have emerged in the form of teas, yogurts, beverages, and wines in China [3], as well as dietary supplements in many Western Countries [2]. So far, only the fruit of *L. barbarum* produced in Ningxia is recorded as the authentic (Daodi) herb in China. However, over the last five years, the demand for *L. barbarum* has dramatically increased in China [2,3]. Therefore, *L. barbarum* has been widely cultivated in different regions of China such as Qinghai, Xinjiang, Inner Mongolia, Hebei, Shanxi, Zhejiang, and Gansu. Generally, water-soluble polysaccharides are considered as one of main bioactive components in *L. barbarum* [2–4]. Therefore, comparison of water-soluble polysaccharides in *L. barbarum* collected from different regions of China is important, which is helpful for their quality evaluation and beneficial for the improvement of their performance in medicinal and functional food area. Recent studies have showed that the compositional monosaccharides and types of glycosidic

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linkages (partial acid and various different enzymatic digestions) of water-soluble polysaccharides in the fruit of *L. barbarum* collected from different regions of China are similar [5,6]. However, molecular weights and contents of water-soluble polysaccharides and their fractions from *L. barbarum* in China have seldom been simultaneously determined.

Usually, size exclusion chromatography (SEC) with dextran or pullulan standards as calibration curve has been performed for the determination of molecular weight of polysaccharides in *L. barbarum* [7–9]. However, due to the difference of hydrodynamic volume between dextran or pullulan standards and tested samples [10], the accuracy for SEC with standard curves is poor. In addition, up to date, a few of studies have been performed for the quantification of polysaccharides in wolfberry collected from different regions of China and various cultivars using phenol sulfuric acid assay with glucose as reference standard [6,11,12]. However, the phenol sulfuric acid assay always has poor specificity and accuracy due to the complex compositional monosaccharides of natural polysaccharides [13]. Moreover, the phenol sulfuric acid assay is not available for the simultaneous determination of the content of polysaccharides and their different fractions. Actually, bioactivities of polysaccharides from natural resources are correlated to their molecular weights [14,15] and contents [2]. Therefore, accurate quantification of different water-soluble polysaccharide fractions in wolfberry is necessary and important, which is helpful for the improvement of their performance in the medicinal and functional food area, and beneficial to improve the quality control of *L. barbarum*.

SEC coupled with multi-angle laser light scattering (MALLS) and refractive index detector (RID) has been demonstrated to be a powerful and typical technique for the determination of the absolute molecular weight, particle size, and chain conformation of polysaccharides [16,17]. More recently, a HPSEC-MALLS-RID with the universal refractive index increment (*dn/dc*) method, which can be utilized for the direct determination of contents based on the concentration-specific refractive index increment equation without reference standard, has also been developed and applied for quantitative analysis of polysaccharides and their fractions from *Panax* spp. in our lab [18]. Therefore, in this study, in order to accurately determine the molecular mass and contents of polysaccharides and their fractions in *L. barbarum*, and evaluate the quality of *L. barbarum* collected in China, the molecular mass and contents of different water-soluble polysaccharide fractions in fifty batches of *L. barbarum* collected from different regions of China were simultaneously determined using HPSEC-MALLS-RID with the *dn/dc* method.

2. Materials and methods

2.1. Materials and chemicals

Fifty batches of fruits of *L. barbarum* (LB01 to LB50) were collected from different regions of China in 2013 and 2014, respectively (Table. 1). The samples of *L. barbarum* were identified by Professor Shao-Ping Li, University of Macau, Macau SAR, China, for authentication of their species based on the survey of their plants in cultivation base, and the macromorphologic characters of the fruits, and the species of these samples were also further confirmed by the National Institutes for Food and Drug Control of China. The voucher specimens (Fig. 1) were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

Dextran 25 K, dextran 270 K, dextran 410 K and bovine serum albumin (BSA, M_W = 66 kDa) were purchased from Sigma (St. Louis, MO, USA). Deionized water was prepared by the Millipore Milli Q-Plus system (Millipore, Billerica, MA, USA). All other reagents were of analytical grade.

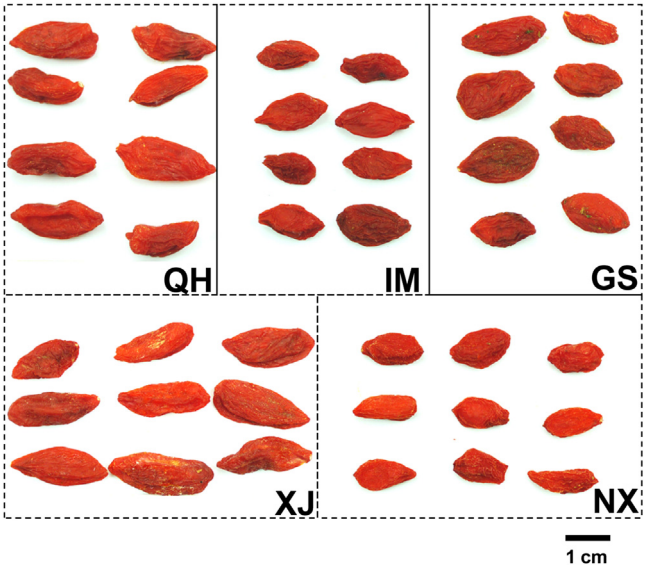

Fig. 1. The typical materials of *Lycium barbarum* collected in China.
QH, IM, XJ, GS, and NX, *L. barbarum* collected from Qinghai, Inner Mongolia, Xinjiang, Gansu, and Ningxia in China, respectively.

2.2. Preparation of water-soluble polysaccharides from L. barbarum

The samples were grounded into a fine powder, and dried by freeze-drying for 48 h. Sample materials (2.0 g) were immersed in 80% (*v*/*v*) of methanol (30.0 mL) and refluxed in a Syncore parallel reactor (Büchi, Flawil, Switzerland) for 1.0 h at 65 °C with stirring at 150 rpm according to a previous reported method [5]. And the extract solution was centrifuged at 4000*g* for 10 min (Allegre X-15 centrifuge; Beckman Coulter, Fullerton, CA, USA), the supernatant was removed. The methanol extracted residues were then dried under vacuum at 45 °C for 4 h, and water-soluble polysaccharides were extracted using microwave assisted extraction according to a previous reported method [5]. In brief, the dried methanol extracted residues (~2.0 g) were suspended in 40 mL of deionized water and extracted with microwave assisted extraction (Multiwave 3000, Anton paar GmbH, Graz, Austria). The extraction process was performed at different microwave power (700, 900, 1100, and 1300 W) and irradiation time (5, 7, 9, and 11 min) to obtain an optimal extraction condition. Then the extract was centrifuged at 4000*g* for 10 min. The supernatant (~40.0 mL) was evaporated to 10.0 mL of solution under vacuum using rotary evaporator (Büchi, Flawil, Switzerland). Subsequently, ethanol (95%, *w*/*v*) was added to the final concentration of 80% (*v*/*v*) for precipitation of crude polysaccharides. The precipitate was redissolved in 10.0 mL of hot water. After centrifugation (4000*g* for 10 min), the supernatant was collected and made up to 10.0 mL, and molecular weights and contents of water-soluble polysaccharides were determined by using HPSEC-MALLS-RID with the *dn*/*dc* method. In addition, the extraction recovery of microwave assisted extraction under optimal conditions was investigated. Briefly, after the first extraction, the extracted residues were thoroughly washed with water. Then the same volume of water was added and extracted again under the same condition as described above.

Finally, the water-soluble polysaccharides in all samples were extracted and prepared in duplicates under the optimal extraction conditions for further analysis.