International Journal of Biological Macromolecules 78 (2015) 230–237

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Fingerprinting profile of polysaccharides from *Lycium barbarum* using multiplex approaches and chemometrics

Wei Liu[a,b,1], Jinnan Xu[a,b,1], Rui Zhu[a,b], Yiqing Zhu[a,b], Yang Zhao[c], Pei Chen[c], Chun Pan[d], Wenbing Yao[a,b,*], Xiangdong Gao[a,b,**]

[a] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China
[b] School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, PR China
[c] Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, United States
[d] Department of Critical Care Medicine, Zhongda Hospital, Southeast University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form 22 March 2015
Accepted 27 March 2015
Available online 3 April 2015

Keywords:
Lycium barbarum
Fingerprinting profile
Chemometrics

ABSTRACT

Techniques including ultraviolet–visible spectra (UV), high performance size-exclusion chromatography (HPSEC), Fourier-transform infrared spectroscopy (FT-IR) and pre-column derivatization high-performance liquid chromatography (PCD-HPLC) were used in the fingerprinting analysis of *Lycium barbarum* polysaccharides (LBPs) from different locations and varieties. Multiple fingerprinting profiles were used to evaluate the similarity and classification of different LBPs with the help of chemometrics. The results indicated that sixteen batches of LBPs had good consistency, and fingerprinting techniques were simple and robust for quality control of LBPs as well as related products. In addition, fingerprinting techniques combined with chemometrics could also be used to identify different cultivation locations of LBPs samples. Finally, four monosaccharides (galacturonic acid, glucose, galactose and arabinose) and the absorptions of stretching vibration of ester carbonyl groups as well as N—H variable angle vibration of —CONH— could be selected as herbal markers to distinguish different samples.

1. Introduction

Lycium barbarum L. (*L. barbarum*) has a variety of pharmacological activities and plays a significant role in prevention and treatment of some chronic diseases including cancer, hepatitis, diabetes, hyperlipidemia, thrombosis, immunodeficiency and male infertility [1,2]. *L. barbarum* products classified as nutraceutical foods or dietary supplements including juice, wine, tea, and conventional food products like yogurts have become popular in East Asia, Europe and North America [3]. As the main supplier of functional *L. barbarum* products, China had total exports reaching US$ 120 million in 2010 [1]. Therefore, the development of rigorous quality control procedures for *L. barbarum* products is urgently needed [4]. Previous studies focused on the varied small molecules including zeaxanthin dipalmitate, carotenes, betaine, flavone and trace elements in different species [5–8]. Besides those small molecules, polysaccharides are also considered as major beneficial components of *L. barbarum* [9,10]. However, there were few reports on the differentiation and quality control of *L. barbarum* polysaccharides (LBPs) from different varieties or locations because of their complicated structural features. Therefore, the quality control has been the main issue in functional polysaccharide products commercialization process [11].

Fingerprinting techniques have been proven to be effective and convenient for quality control and standardization of various herbal materials, especially when authentic standards are not available [12,13]. It was reported that fingerprinting techniques have been used in characterization, discrimination and quality control of polysaccharides from *Cordyceps*, *Dendrobium*, *Ganoderma*, and tea products [11,14–16], indicating that the techniques served as effective tools for quality control and standardization of functional polysaccharides.

Unlike small molecule compounds, it is difficult to describe a polysaccharide only by content determination or one specific spectrum. Structural features including sugar compositions, molecular

* Corresponding author at: State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China. Tel.: +86 25 83271218; fax: +86 25 83271218.
** Corresponding author at: School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, PR China. Tel.: +86 25 83271543; fax: +86 25 83271249.
E-mail addresses: wbyao@cpu.edu.cn (W. Yao), xdgao@cpu.edu.cn (X. Gao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijbiomac.2015.03.062
0141-8130/

weight distribution, and glycosidic bond types are all needed for comprehensive description of polysaccharide products. Therefore, it is very important to build a rapid and reliable multi-fingerprinting analysis model to identify the variance of *L. barbarum* polysaccharides (LBPs) based on all these structural features. In recent years, attention has been paid on chemometrics generally used to analyze multi-component systems due to fast and large amount of data collection with HPLC, IR and NMR [11,17,18]. Chemometrics is a family of multivariate statistical methods that are applied to chemical data [19]. Therefore, the most common methods used to evaluate chromatographic fingerprinting data sets, such as similarity analysis (SA), principal component analysis (PCA), artificial neural networks, k-nearest neighbor, hierarchical cluster analysis (HCA) and partial least square-discriminant analysis (PLS-DA), and so on, were comprehensively studied [20,21].

In this study, polysaccharides from sixteen *L. barbarum* samples with different varieties or locations were firstly analyzed through fingerprinting techniques, including ultraviolet–visible spectra (UV), high performance size-exclusion chromatography (HPSEC), Fourier-transform infrared spectroscopy (FT-IR) and pre-column derivatization high-performance liquid chromatography (PCD-HPLC) based on their structural features. Similarity of the samples and the herbal markers that played the key role in similarity were obtained with the aid of chemometrics. The results served as scientific basis to evaluate the quality of *L. barbarum* related products. Moreover, places of origin which produced similar *L. barbarum* fruits with Ningxia could also be found based on the results of chemometric analysis on the fingerprinting data.

2. Materials and methods

2.1. Materials and reagents

Sixteen batches (S1–S16) of *L. barbarum* samples were collected from different locations in China (Table 1). Among them, S1 (NQX), S8 (NQ1), S9 (NQ2), S10 (NQ4), S11 (NQ5) and S12 (NQ7) were all obtained from the Research Institute of Agriculture and Forestry in Ningxia Hui Autonomous region, China, and they were Ningqi #1 rich in selenium, common Ningqi #1, Ningqi #2, Ningqi #4, Ningqi #5 and Ningqi #7, respectively. They were cultivated varieties of *L. barbarum* and studied as representative varieties of *L. barbarum* [22–24]. Among them, NQ1, NQ2 and NQ4 were the varieties of *L. barbarum* by single plant breeding [25], while NQ5 was bred from male-sterile NQ1 *L. barbarum* (YX-1) by asexual plant propagation [26], and NQ7 was also another natural variation of NQ1 *L. barbarum* [27]. All these samples were identified as *L. barbarum* by Prof. Minjian Qin from School of Traditional Chinese Medicine.

N-Acetyl-D-glucosamine (GlcNAc) was purchased from Aladdin Industrial Co. (Shanghai, China), and D-glucosamine hydrochloride was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Dextrans were purchased from the National Institute for the Control of Pharmaceutical and Biological products (Beijing, China). D-Mannose (Man), D-ribose (Rib), L-rhamnose (Rha), D-glucuronic acid (GlcUA), D-galacturonic acid monohydrate (GalUA), D-glucose (Glc), N-acetyl-D-galactosamine (GalNAc), D-galactose (Gal), D-xylose (Xyl), L-arabinose (Ara), L-fucose (Fuc) and bovine serum albumin (BSA) were obtained from Sigma–Aldrich Trading (St. Louis, MO, USA). Coomassie brilliant blue G-250, phenol, sulfuric acid, trifluoroacetic acid (TFA) and potassium bromide (KBr) were purchased from Nanjing chemical reagent Co. Ltd. (Nanjing, China). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Acros Organics (Geel, Belgium). Other reagents and chemicals were analytical grade.

2.2. Preparation of L. barbarum polysaccharides

The preparation was according to our previous reported method with proper modifications [28]. Each of the dried fruits of *L. barbarum* was pulverized and the powder (200 g) was immersed into 80% ethanol solution for twice (2 h for each time) to remove small molecule materials and pigment. Then the residue was defatted with chloroform-methanol solution (v/v = 2:1) for another 4 h in a soxhlet extractor. After that, the dry residue was extracted with water at 80 °C for three times (2 h for each time) and the extractions were centrifuged at 4000 rpm for 5 min to remove the solid part. Then the concentrated extract (1:5, v/v) was precipitated by 80% ethanol. The precipitation was collected by centrifugation, washed with ethanol and acetone successively. Finally, crude polysaccharide powder coded as LBPs was dried under reduced pressure. The yield of LBPs was calculated using the following equation:

$$\text{LBPs yield}\%(w/w) = \frac{\text{dried LBPs weight}}{\text{powder weight}(200\,\text{g})} \times 100\%$$

2.3. Analytical methods of components in LBPs

The total carbohydrate content was determined using the phenol-sulfuric acid method [29] as D-glucose equivalents. The *m*-hydroxydiphenyl-sulfuric method was adapted to determine the uronic acid content [30] using galacturonic acid as the standard. The total protein content was determined by the Bradford method [31] with bovine serum albumin (BSA) as a standard.

2.4. UV fingerprints analysis

The LBPs samples (1 mg/mL) in 1.00 cm quartz cell were detected by UV–vis spectrophotometer within the scanning range of 200–400 nm (1.0 nm interval) against the blank of distilled water.

2.5. HPSEC fingerprints analysis

Each sample (10 mg) was dissolved in distilled water (2 mL) and then filtered by a 0.45 μm membrane. 20 μL of the supernatant was applied to Agilent 1100 High Performance Liquid Chromatography with a Shodex SUGAR KS-805 column (8 mm ID × 300 mm, Showa Denko, Japan), each sample ran 20 min and the oven temperature was maintained at 30 °C. The samples detected by a refractive index detector were eluted with deionized water at the rate of 1.0 mL/min.

2.6. FT-IR fingerprints analysis

The samples were ground into powder and pressed into 1 mm pellets with spectroscopic grade KBr for FT-IR determination by a BRUKER-MPA spectrophotometer in the range of 4000–400 cm^{-1} with a resolution of 2 cm^{-1}.

2.7. PCD-HPLC fingerprints analysis

16 batches of LBPs (5 mg) were dissolved and hydrolyzed using 2 mol/L trifluoroacetic acid solution (TFA, 1 mL) in a boiling water bath for 8 h. Then, TFA was removed absolutely by washing with methanol (1 mL) for four times. The dried hydrolysates were dissolved in ultra-pure water (1 mL). PMP derivatization was conducted as described previously with slight adjustments [32]. Briefly, 13 standard monosaccharides (Man, GlcN, Rib, Rha, GlcUA, GalUA, NAcGlc, Glc, NAcGal, Gal, Xyl, Ara, Fuc) mixture solution (100 μL, 20 mmol/L) and 16 batches of LBPs hydrolyzed samples (100 μL) were dissolved in 0.6 mol/L NaOH solution (50 μL), and 0.5 mol/L PMP methanol solution (100 μL) was added. The samples