Journal of Functional Foods 17 (2015) 903–909

Available online at www.sciencedirect.com

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

Chemical properties and bioactivities of Goji (*Lycium barbarum*) polysaccharides extracted by different methods

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ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 6 June 2015
Accepted 16 June 2015
Available online

Keywords:
Lycium barbarum
Polysaccharides
Subcritical water extraction
Ultrasound
Antioxidant activity
Immunomodulation

ABSTRACT

Lycium barbarum fruit (Goji or wolfberry) is widely used as a functional food as well as an herb in Chinese medicine. The polysaccharides of *L. barbarum* (LBPs) are well-known for immunomodulation and antioxidant activities. This study evaluated the yields, properties and bioactivities of LBPs extracted from *L. barbarum* fruit by different methods including hot water (HWE 100 °C), ultrasonic water (UWE 30–40 °C), subcritical water (SWE 110 °C), and ultrasound-enhanced subcritical water extraction (USWE 110 °C). The total LBP yield (80 min extraction followed by ethanol precipitation) was the highest from USWE (14% w/w) and the lowest from HWE (7.6%). The LBP from USWE also showed the highest protein (27.9%) and phenolic content (5.3%, w/w), and the highest antioxidant activities (scavenging radicals by DPPH and trolox equivalent antioxidant capacity assays; ferric reducing power). It also showed the highest immunoactivities in activating the phagocytosis and NO production of RAW264.7 macrophages. USWE was proven the most efficient method for extraction of LBPs. Temperature and ultrasound were two chief factors affecting the extraction yield, chemical properties and bioactivities of LBP.

1. Introduction

Lycium barbarum fruit, generally called Goji berry or wolfberry, is a well-known herb in traditional Chinese medicine (The Pharmacopoeia of the People's Republic of China). Nowadays, Goji berries are being used not only in China but also worldwide as a popular health food ingredient in various forms such as soups, drinks and a variety of solid foods (Amagase & Farnsworth, 2011; Potterat, 2010). Goji berry has shown a wide range of health benefits such as the functions and activities associated with the liver, kidney, eyesight, sex, immune system, circulation and longevity (Tang et al., 2012). *L. barbarum* polysaccharides (LBPs) represent a major class of bioactive

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http://dx.doi.org/10.1016/j.jff.2015.06.045
1756-4646/

ingredients of Goji berries which have multiple health effects such as immunomodulation (Gan, Zhang, Liu, & Xu, 2003), anticancer (Gan, Zhang, Yang, & Xu, 2004) and antioxidant activities (Lin, Wang, Chang, Inbaraj, & Chen, 2009; Niu, Wu, Yu, & Wang, 2008).

Extraction is the first and essential step for characterization and utilization of the bioactive polysaccharides (PS) from the plant materials. Hot water extraction (HWE) is a common method for extraction of LBPs and other water-soluble constituents from Goji berries in laboratory and industry. However, HWE is rather time-consuming, energy-intensive and relatively inefficient in large-scale production. Alternative extraction methods have been developed and applied to improve the conventional extraction, such as the application of power ultrasound and high pressure. Ultrasound assisted extraction (UAE), the application of high-intensity ultrasound to the extraction liquid, has been widely used to improve the water or solvent extraction of natural products from various sources (Vilkhu, Mawson, Simons, & Bates, 2008). Subcritical water or pressurized hot water refers to liquid water heated under pressure to a temperature higher than the normal boiling temperature (100 °C) but below the critical temperature (374 °C). Subcritical water extraction (SWE) has been successfully applied to extract organic pollutants from environmental samples (Schantz, 2006) and bioactive ingredients from natural products (Ong, Cheong, & Goh, 2006). However, it is difficult to apply mechanical agitation in the pressurized extraction vessel to improve the mass transfer in the solid and liquid phase. To overcome this problem, Zhao, Dong, Chen, and Hu (2010) constructed an ultrasound-enhanced subcritical water extraction (USWE) by placing an ultrasonic probe into the SWE vessel and applied to extraction of polysaccharides from the Goji berries (Zhao et al., 2010).

This study evaluated the yields, properties and bioactivities of LBPs attained from Goji berries by USWE and three other methods, HWE, SWE and ultrasonic water extraction (UWE). The chemical properties of LBPs were characterized by total carbohydrate, protein and phenolic contents, and molecular weight profiles, and the bioactivities by antioxidant activities and *in vitro* immunomodulatory activities.

2. Materials and methods

2.1. Chemicals and biological materials

Reagents for ferric reducing antioxidant power (FRAP) and ABTS (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) assays were obtained from Beyotime Institute of Biotechnology (Nantong, Jiangsu, China). All other analytical and assay agents, and standards, such as ethanol, phenol, dextrose, Folin–Ciocalteu phenol reagent, copper sulphate, bovine serum albumin, gallic acid, dextran molecular weight standards, 1,1-diphenyl-2-picrylhydrazyl (DPPH), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of sufficient purity grades for the required uses.

Dried *L. barbarum* Goji berries, originated from Ningxia, China, were provided and certified by the Research & Development Centre of Infinitus (China) Co. Ltd. Upon arrival, the fruits in plastic zipper bags were stored at 4 °C before use. RAW264.7 cell line was attained from American Type Culture Collection (Manassas, VA, USA), and the culture medium RPMI 1640, trypsin, foetal bovine serum (FBS), streptomycin and penicillin were purchased from Gibco (Grand Island, NY, USA). Lipopolysaccharide (LPS), toluylene red solution and MTT assay reagents were obtained from Sigma Chemical Co. NO assay kit was bought from Juli Biological Medical Engineering Institute (Nanjing, China).

2.2. Extraction and isolation of LBPs

The Goji berries were dried in an oven at 60 °C to constant weight and ground into powder with an electrical mill. Each 10 g of the solid powder was mixed with 300 mL of de-ionized (DI) water (1:30 solid/liquid ratio) and extracted with four different methods as follows. Hot water extraction (HWE) was performed in boiling water at ~100 °C. For ultrasonic extraction (UE), the fruit–water mixture was treated with an ultrasonic homogenizer (360 W) at room temperature. Subcritical water extraction (SWE) was performed at 110 °C and 5 MPa. In ultrasound-enhanced SWE (USWE), the mixture was sonicated with an ultrasonic processor (160 W) at 110 °C and 5 MPa. After extraction for 80 min, the extract solution was separated from the solid residue by filtration and then concentrated to about 100 mL by evaporation. Ethanol (95% grade) was slowly added at 4:1 volume ratio to the concentrated extract solution, and the mixture was left for 12 h at 4 °C. The resultant precipitate was collected, and successively washed with anhydrous ethanol and acetone, and then freeze dried, yielding the crude LBP fractions, designated LBP-H from HWE, LBP-U from UE, LBP-S from SWE, or LBP-US from USWE.

2.3. Analysis of LBP composition and molecular weight

The total sugar content of LBPs was determined by the phenol-sulphuric acid method using glucose as a reference (Cheung, Siu, & Wu, 2012). Total protein content was determined by Lowry method as described using bovine serum albumin (BSA) as a reference (Wang, Chang, & Chen, 2009). Total phenolic content was determined by Folin–Ciocalteu assay reagent using gallic acid as a reference (Siu, Chen, & Wu, 2014).

Molecular weight (MW) distribution of LBPs was analysed by high performance gel permeation chromatography (HPGPC) with the instrument system and conditions as reported by Chen, Ding, Wang, Siu, and Wu (2014). In brief, the HPGPC system consisted of a Waters 1515 isocratic HPLC pump, a Waters 2414 refractive index (RI) detector and a Waters 2998 UV detector (Chen et al., 2014). Two GPC columns were used in series, Ultrahydrogel™ 2000 and Ultrahydrogel™ 500 columns. Mobile phase consisted of 2.9 g/L $NaH_2PO_4.2H_2O$ and 11.5 g/L Na_2HPO_4 at a flow rate of 0.6 mL/min. MW was calibrated with dextran standards ranging from 5 to 670 kDa.

2.4. Antioxidant activity assays

Antioxidant activities of LBPs were determined by three chemical assays, ferric reducing ability of plasma (FRAP), trolox equivalent antioxidant capacity (TEAC) for scavenging ABTS radicals, and DPPH radical scavenging assay as described