Carbohydrate Polymers 124 (2015) 201–207

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

An evaluation system for characterization of polysaccharides from the fruiting body of *Hericium erinaceus* and identification of its commercial product

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ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form 5 February 2015
Accepted 6 February 2015
Available online 21 February 2015

Keywords:
Hericium erinaceus
Polysaccharide
HPSEC–MALLS–RID
GC–MS
Saccharide mapping
PACE

ABSTRACT

An evaluation system including colorimetric assay with iodine and potassium iodide, HPSEC–MALLS–RID analysis, GC–MS analysis, and saccharide mapping based on PACE analysis was proposed for the identification and discrimination of commercial product of *Hericium erinaceus* based on the chemical characters of polysaccharides in *H. erinaceus* fruiting body collected from different regions of China. The results showed that the molecular weights, the compositional monosaccharides and the glycosidic linkages of polysaccharides in *H. erinaceus* collected from different regions of China were similar, respectively. However, polysaccharides in the widely consumed product of *H. erinaceus* in China were significantly different from those of *H. erinaceus* fruiting body. The implications from these results were found to be beneficial to improve the quality control of polysaccharides from the *H. erinaceus* fruiting body, and suggest that the proposed evaluation system could be used as a routine approach for the quality control of polysaccharides in other edible and medicinal mushrooms.

1. Introduction

Hericium erinaceus, is a well-known edible and medicinal mushroom (Fig. 1), which belongs to the Aphyllophorales, Hydnaecase, and Hericium families. It has been used as a tonic food, and a traditional Chinese medicine for the prevention and treatment of gastric ulcers, chronic gastritis and other digestive tract-related diseases in East Asian (Li et al., 2014). Recently, *H. erinaceus* has attracted a great deal of attention in the biomedical and functional foods science due to its various beneficial effects (Khan, Tania, Liu, & Rahman, 2013), and a large amounts of *H. erinaceus* products (polysaccharides, etc.,) are consumed in China. Usually, polysaccharides are considered as one of the major bioactive components with the highest content in *H. erinaceus* (Jiang, Wang, Sun, & Zhang, 2014; Zhu, Li, et al., 2014), which possess various pharmacological properties such as antioxidant (Han, Ye, & Wang, 2013; Li, Wang, Wang, Walid, & Zhang, 2012; Zhang, Lv, et al., 2012), immunomodulatory (Lee, Cho, & Hong, 2009; Sheu, Lyu, Lee, & Cheng, 2013), antitumor (Kim, Kang, Kim, Nam, & Friedman, 2011; Lee & Hong, 2010), hepatoprotective (Zhang, Lv, et al., 2012), anti-*Helicobacter pylori* (Zhu, Chen, et al., 2014) and antibacterial activities (Kim, Moon, Nam, & Friedman, 2012). Indeed, the bioactivities of polysaccharides are closely correlated with their physico-chemical properties such as molecular weight, compositional monosaccharides and glycosidic linkages (Li, Wu, Lv, & Zhao, 2013). However, polysaccharides from the fruiting body of *H. erinaceus* and its commercial products have seldom been compared due to the lack of an efficient and reliable strategy. Therefore, a reliable and efficient strategy or evaluation system for characterization and comparison

Abbreviations: ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; GC–MS, gas chromatography mass spectrometry; HPSEC, high-performance size-exclusion chromatography; MALLS, multi angle laser light scattering; PACE, polysaccharide analysis using carbohydrate gel electrophoresis; RID, refractive index detection; SMC, simulative mean chromatogram.

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http://dx.doi.org/10.1016/j.carbpol.2015.02.028
0144-8617/

Fig. 1. The cultivated fruiting body of *H. erinaceus*.

of polysaccharides from *H. erinaceus* as well as identification of its commercial products is urgently required, which is beneficial to improve their quality control and performance in the biomedical and functional foods area.

High performance size exclusion chromatography coupled with multi angle laser light scattering and refractive index detection (HPSEC–MALLS–RID) has been widely used for characterization and comparison of bioactive polysaccharides from natural resources such as *Gymnadenia conopsea* (Lin, Wu, Xie, Zhao, & Li, 2015) and *Cordyceps sinensis* (Wu, Meng, et al., 2014), which has been proven as one of the most powerful techniques for the investigation of the molecular weight of polymers. In addition, saccharide mapping based on polysaccharide analysis using carbohydrate gel electrophoresis (PACE) has also been widely employed for analysis of the structural characters (glycosidic linkages, etc.,) of polysaccharides from *Ganoderma* spp. (Wu, Xie, Hu, Zhao, & Li, 2013) and *Cordyceps* spp. (Wu, Cheong, et al., 2014; Wu, Xie, et al., 2014), respectively, which has been proven to have high repeatability, stability, and throughput. Furthermore, gas chromatography–mass spectrometry (GC–MS) analysis is a feasible and desirable technique for qualitative and quantitative analysis of compositional monosaccharides released from polysaccharides (Li et al., 2013), which has been commonly used for the structural characterization of polysaccharides from medicinal plants and fungi (Hu, Cheong, Zhao, & Li, 2013). Therefore, in this study, an evaluation system including colorimetric assay with iodine and potassium iodide, HPSEC–MALLS–RID, GC–MS, and saccharide mapping based on PACE analysis was proposed for identification and discrimination of commercial product of *H. erinaceus* based on the chemical characters of polysaccharides in *H. erinaceus*.

2. Materials and methods

2.1. Materials and chemicals

Five batches (HE1–HE5) of *H. erinaceus* fruiting body were collected from different regions of China, and one batch of widely consumed water extracts (mainly contains polysaccharides, WEP) of *H. erinaceus* was collected from Guangdong Province, China (Table 1). Identity of the *H. erinaceus* was confirmed by Doctor Chun-Feng Qiao, University of Macau, Macau SAR, China. The voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

D-Glucose, α-amylase (EC 3.2.1.1), pectinase (EC 3.2.1.15), dextranase (EC 3.2.1.11), dextran (DEN), starch (ST) and acetic anhydride were purchased from Sigma (St. Louis, MO, USA). Laminaribiose (Lam2, 95%), laminaritriose (Lam3, 95%), and laminaritetraose (Lam4, 95%), β-1,3-D-glucan (GLN), pectic galactan (PGN), polygalacturonic acid (PGA), β-1,3-D-glucanase (EC 3.2.1.39), and β-1,4-D-galactanase (EC 3.2.1.89) were purchased from Megazyme (Wicklow, Ireland). 8-Aminonaphthalene-1,3,6-trisulfonic acid (ANTS) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Polyacrylamide containing a ratio of acrylamide/N,N-methylenebisacrylamide (19:1, w/w) was obtained from Bio-Rad (Hercules, CA, USA). Deionized water was prepared by a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade.

2.2. Preparation and colorimetric analysis of polysaccharides

Hot water extraction was performed according to a previously reported method with minor modification (Wu, Xie, et al., 2014). Briefly, the samples were dried by freeze-drying for 24 h, and pulverized. Sample materials (1.0 g) were immersed in water (20.0 mL) and refluxed in a Syncore parallel reactor (Büchi, Flawil, Switzerland) for 1.5 h at 100 °C with stirring at 150 rpm, respectively. Then the extract solution was centrifuged at 4000 × *g* for 10 min (Allegre X-15 centrifuge; Beckman Coulter, Fullerton, CA, USA). 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 mL of hot water (60 °C). After centrifugation (4500 × *g* for 15 min), the supernatant was collected and the powder of the supernatant was obtained by freeze-drying. Finally, the crude polysaccharides were prepared in duplicates for further analysis.

Usually, polysaccharides from the edible and medicinal mushrooms have negative response with the reagent of iodine and potassium iodide. However, the additives such as soluble starch and maltodextrin are usually added into the commercial water extracts (WEP) of *H. erinaceus*. Therefore, in order to determine the additives in WEP, polysaccharides from the fruiting body of *H. erinaceus* and WEP were firstly compared using colorimetric assay with iodine and potassium iodide.

2.3. HPSEC–MALLS–RID analysis

The molecular weight (M_w) and the polydispersity index (PDI, M_w/M_n) of polysaccharides and their different fractions from *H. erinaceus* and its commercial product were measured using HPSEC–MALLS–RID according to a previous reported method with minor modification (Chen et al., 2013; Lin et al., 2015). In brief, HPSEC–MALLS–RID measurements were carried out on a multi-angle laser light scattering (DAWN HELEOS, Wyatt Technology Co., Santa Barbara, CA, USA) with an Agilent 1100 series LC/DAD system (Agilent Technologies, Palo Alto, CA, USA) equipped with columns of TSK-Gel G6000PW$_{XL}$ (300 mm × 7.8 mm, i.d., Tosoh Bioscience, Tokyo, Japan) and TSK-GEL G3000PW$_{XL}$ (300 mm × 7.8 mm, i.d.) in series at 35 °C. A refractive index detector (RID, Optilab rEX refractometer, DAWN EOS, Wyatt Technology Co., Santa Barbara, CA, USA) was simultaneously connected. The MALLS instrument was equipped with a He–Ne laser (λ = 658 nm). The M_w was calculated by the Zimm method (Zimm, 1948). The *dn*/*dc* value of polysaccharide from *H. erinaceus* in 0.9% NaCl aqueous solution was measured by using a refractive index detector at 658 nm and 35 °C to be 0.131 ± 0.003 mL/g. The mobile phase was 0.9% NaCl aqueous solution at a flow rate of 0.5 mL/min. Each sample was dissolved in 0.9% NaCl aqueous solution at a final concentration of about 2.0 mg/mL, then filtered through a 0.45 μm membrane before use. The injection volume was 50 μL. The Astra software (Version 6.0.2, Wyatt Tech.