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Advanced analysis of polysaccharides, novel functional components in food and medicine dual purposes Chinese herbs

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ABSTRACT

Polysaccharides with multiple biological activities and health benefit effects are usually considered as one of the major function components in food and medicine dual purposes Chinese herbs (FMDPCH). With the increasing interest in the utilization of FMDPCH, their quality control is very important for ensuring their safety and efficacy. To date, there is no report focus on the analysis of polysaccharides in FMDPCH. Herein, advanced development for quality control approaches, including sample preparation, separation and fraction, qualitative and quantitative analysis, of polysaccharides in MPDPCH were reviewed and discussed. Especially, discrimination and quantification of polysaccharides, respectively, based on novel saccharide mapping and universal refractive index increment were focused. Besides, the perspective for the characterization of polysaccharides has also been described.

1. Introduction

The concept of "food and medicine derived from the same source" was widely accepted for thousand years in China. Similarly, "Let food be thy medicine and medicine be thy food" was also espoused by Hippocrates, the father of modern medicine, nearly 2500 years ago.

Indeed, it is well know that herbal diets can reduce the risk of multiple diseases, and their "phytochemicals" are usually beneficial to the health. Therefore, phytochemical analysis is very important for ensuring their safety and health benefits. In 2011 and 2015, we summarized, respectively, the advanced development during the periods of 2006–2010 and 2011–2014 in analysis of phytochemicals from food and medicine dual-purposes Chinese herbs (FMDPCH) used in China [1,2], which mainly focused on the small functional molecules.

Polysaccharides are often considered as food source and structural building block. Their biological roles have in many ways been overlooked in the past due to their unique complexity. Nevertheless, over the last decade, researchers have increasingly turned their attention toward understanding the role of polysaccharides in normal cellular function and in disease, which opened up new research fronts in terms of probing glycans as targets in the design and development of novel drugs or new therapies for infectious disease, neurological disease, cancer, and metabolic disorders. Indeed, polysaccharides have been considered as novel important functional components for their significant biological activities,

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); AF4, asymmetrical flow field-flow fractionation; ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt; APTS, 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt; ATPE, aqueous two-phase extraction; ATPS, aqueous two-phase system; CE, capillary electrophoresis; DMSO, dimethyl sulfoxide; *dn*/*dc*, universal refractive index increment; DONS, diffusion-ordered NMR spectroscopy; ELSD, evaporative light scattering detector; ESI-MS, electrospray ionization mass spectrometry; FMDPCH, food and medicine dual purposes Chinese herbs; GC, gas chromatography; HILIC, hydrophilic interaction liquid chromatography; HMM, high-molar mass; HPAEC, high performance anion exchange chromatography; HPGPC, high performance gel permeation chromatography; HPLC, high performance liquid chromatography; HPTLC, high performance thin layer chromatography; HSCCC, high-speed countercurrent chromatography; LC-MS, liquid chromatography-mass spectrometry; LOD, limits of detection; LOQ, Limits of quantification; LS, light scattering; MAATPE, microwave-assisted aqueous two-phase extraction; MAE, microwave-assisted extraction; MALDI, matrix-assisted laser desorption/ionization; MALLS, multi-angle laser light scattering; M_w, molecular weight; MWD, molecular weight distribution; NMR, nuclear magnetic resonance; OLIMP, oligosaccharide mass profiling; ORAC, oxygen radical absorbance capacity; PACE, polysaccharide analysis using carbohydrate gel electrophoresis; PMP, 1-phenyl-3-methyl-5-pyrazolone; Q-TOF, quadrupole time-of-flight; RID, refractive index detector; SEC, size exclusion chromatography.

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such as antioxidation, immune potentiation, antitumor, antiinflammation and blood sugar reduction (Supplemental Table 1). Actually, the biological activities of polysaccharides are closely correlated to their physicochemical properties [3]. Therefore, characterization of polysaccharides is necessary. Unfortunately, glyco-analysis is a big challenge due to the complexity of polysaccharides. Though, some techniques for carbohydrate analysis were review in last years [3–5], quality control approaches, including sample preparation, separation and fraction, qualitative and quantitative analysis, of polysaccharides in MPDPCH was never emphasized, which will be reviewed and discussed in this review.

2. Sample preparation

Sample preparation is the crucial first step in the analysis of polysaccharides from Chinese medicines because it can greatly influence the fraction and composition. Because of very high polarity, hot water decoction is the commonly used method for extraction of polysaccharides. It also usually requires a combined use of other processes such as hydrolysis (acids [6–8] and enzymes [9–13]), chemical derivatization [14] or physical treatments [15,16], in order to obtain polysaccharides with specific structure characteristics and higher bioactivities. An optimal citric acid extraction condition was developed to obtain polysaccharide from *Laminaria japonica*. The viscosity of polysaccharide extracted by citric acid (LJPA) was eight times lower than that of polysaccharide extracted by hot water (LJPW), which exhibited significantly higher antioxidant capacities including oxygen radical absorbance capacity (ORAC), ABTS radical scavenging activity and reducing power than LJPW. Citric acid extraction showed a positive influence on the polysaccharide degradation and antioxidant capacities of *L. japonica* [6]. Generally, before water extract, organic solvents, including mixture of chloroform–methanol [17,18], ether [18], petroleum ether [19,20], methanol [21], different concentration of ethanol [21–25], are used for removing the interfering components such as some colored materials, lipids, small molecule materials of monosaccharide, disaccharide and oligosaccharide. The parameters for extraction, such as pH, temperature, extraction time and the ratio of water, could be optimized using single factor experimental design [26], orthogonal test design [19,20,27–33], Box-Behnken experimental design [32,34,35] and response surface methodology [29,34,36–53]. Ultrasonic extraction (UAE) [32,36,52–55], UAE with enzymatic digestion [41,56] also can be employed for improving the extraction efficiency and specificity. Box-Behnken experimental design and response surface methodology were used to optimize UAE conditions of simultaneous extraction for flavonoids and polysaccharides from *Chaenomeles sinensis* (Thouin) Koehne. The optimal extraction conditions were ultrasonic power of 480 W, extraction time of 35 min, extraction temperature of 73°C and material liquid ratio of 1:90 (g/mL). Under these optimal conditions, the total yield of flavonoids and polysaccharides was up to 15.62%, an increase of 21.46% when compared with hot water extraction [34]. However, more attention should be paid to ultrasonication induced degradation of polysaccharides. A water-insoluble polysaccharide (PCS) obtained by alkaline extraction from the sclerotium of a medicinal mushroom, *Poria cocos* was partially depolymerized by ultrasonication for various period of time to obtain two mushroom polysaccharides treated for 10 and 90 min (PCS10 and PCS90). Compare to the untreated polysaccharide (PCS0), PCS90 had a lower intrinsic viscosity (about 30% of PCS0) and M_w (43 kDa) than PCS0. The 13C NMR spectra of PCS0 and PCS90 indicated that they both had a linear (1 → 3)-β-D-glucan which was further confirmed by methylation analysis. TEM results also showed that ultrasonic treatment could open up the compact structure of PCS into linear chain [16]. Water extraction and precipitation with ethanol is the conventional method for isolation and purification of polysaccharides from Chinese herbs [17–24]. The Sevag method has to be utilized to remove the protein impurities in polysaccharides because both proteins and polysaccharides are insoluble in ethanol. Unfortunately, the Sevag method is very inefficient, complicated, and time-consuming, and organic solvents (butyl alcohol and chloroform) are used in the method also leading to concerns about the purity of the resultant product as well as environment. Proteolytic enzymes produced during fermentation were used to remove the protein component of culture media. Proteins present in lily bulb extract were removed by extracellular proteases secreted by *Saccharomyces cerevisiae* during fermentation. This novel method differs from traditional protein removal methods. When lily bulb extract was cultured with *S. cerevisiae* under optimum conditions, the lily polysaccharides yield and the protein removal ratio were 6.56% and 91.46%, respectively [57]. Compared to the Sevag method, fermentation is an eco-friendly method with mild reaction conditions which is relatively easy to operate.

Besides the methods mentioned above, some novel methods, including microwave extraction [29,58–61], pressurized liquid extraction [62], ultrasound-enhanced subcritical water extraction [13,63–66], supercritical fluid extraction continuous fractionation [67], and induced electric fields [68] have also been employed for extraction polysaccharides so as to improve the extraction efficiency. A novel and rapid method for simultaneous extraction and separation of the different polysaccharides from Semen Cassiae (SC) was developed by microwave-assisted aqueous two-phase extraction (MAATPE) in a one-step procedure [60]. Using ethanol/ammonium sulfate system as a multiphase solvent, the effects of MAATPE on the extraction of polysaccharides from SC such as the composition of the aqueous two-phase system (ATPS), extraction time, temperature and solvent-to-material ratio were investigated. Under the optimum conditions, the yields of polysaccharides were 4.49% for the top phase, 8.80% for the bottom phase and 13.29% for total polysaccharides, respectively. Compared with heating solvent extraction and ultrasonic assisted extraction, MAATPE exhibited the higher extraction yields in shorter time. Fourier-transform infrared spectra showed that two polysaccharides extracted from SC in the top and bottom phases by MAATPE were different from each other in their chemical structures. Fig. 1 illustrates a schematic diagram about MAATPE extraction. In the extraction process, powder sample is immersed in the bottom phase. In fact, the extraction is a multiphase process of mass transfer from the herb matrix to the liquid phase and from the bottom phase to the top phase, which integrating MAE with ATPE (aqueous two-phase extraction) bring about a perfect combination of the specific effect of microwave and the selectivity of the ATPS [60]. Therefore, it not only effectively intensified the extraction of polysaccharides with higher yields, but also simultaneously separated the ethanol-soluble and water-soluble polysaccharides from each other. It is a green, efficient and promising alternative to simultaneous extraction of polysaccharides from herbs. The methods used for extraction of polysaccharides from herbs were summarized in Supplemental Table 1.

3. Separation and fraction

Size-exclusion chromatography (SEC) and ion-exchange cellulose chromatography are the most popular approaches for separation and fraction of polysaccharides based on their size or charge, respectively. The serial combination of multiple SEC columns allows broad molecular weight distribution, and provides higher resolution. The advanced development of SEC is coupled with online detection of light-scattering detectors, viscometers, as well as mass spectrometers [4]. However, SEC only can be used for the