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Preparation, structure analysis and ACE inhibitory activity of konjac oligosaccharide

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ABSTRACT

Konjac contains a large amount of glucan, which is a new functional sugar. Konjac oligosaccharides have high medicinal value, and it is of certain significance to prepare konjac oligosaccharides with konjac as raw material. In this study, the crude polysaccharide was extracted from konjac and then isolated by 95% alcohol-precipitation. The crude polysaccharide was degraded with β-mannanase to obtain the degradation product, named KPD. Then the KPD was acetylated by the pyridine acetic anhydride method in order to be purified by silica gel column to obtain the acetylated product named AC-P3. The structure of AC-P3 was analysed by physicochemical and instrumental analyses. The results indicated that the molecular weight of the AC-P3 was 1254 Da, and that the specific rotation was −20°. And then the AC-P3 was deacetylated with CH_3ONa-CH_3OH solution to obtain the final product, named P3. The structure of P3 was also analysed by physicochemical and instrumental analyses. The molecular weight of the P3 was 666 Da, and the specific rotation was +45°. The result of monosaccharide composition revealed that P3 was consisted of mannose (Man), glucose (Glc), and galactose (Gal) with a molar ratio of 2:1:1. Moreover, the NMR characteristic spectras of P3 was compared with that of AC-P3 to conduct the structural characterization of P3. The results indicated that P3 was a kind of tetrasccharide with a linear backbone of Glc-(1→4) Man (1→4) Man and glycosyl residues of P3 was Man (3→1) branched-Gal. The Angiotensin I-Converting Enzyme (ACE) inhibitory activity assay showed that P3 had a certain inhibition on ACE activity.

1. Introduction

Konjac is a species of *Araceae*, whichis a kind of Chinese classical medicine mainly growing in China's Yunnan, Sichuan, Hubei and other regions (Sim et al., 2011). Konjac has many functions, such as lipid lowering, detoxification, anti-cancer, and so on. Glucomannan was the main ingredient of konjac, which accounts for 55%–80% (Nishinari et al., 1992). Konjac glucomannan is a heteropolysaccharide that is composed of β-1,4 glycosidic bonds formed by β-D-glucose and β-D-mannose in a molar ratio of 2: 3 or 1: 1.6. Reports indicated that its backbone was consisted of mannose and branched by β-1,3 bonds at the C3 position (Rhei et al., 1984; Li et al., 2013; Shmahara et al., 1975). Konjac glucomannan is divided into two types, namely, homo-mannan and isomannan (Puchart et al., 2004). Konjac glucomannan has characteristics of colorless, odorless, non-toxic, high viscosity, good thickening, good film forming, good gelation and good emulsification (Yang et al., 1998; Xiao et al., 2017).

Hypertension is a chronic disease of elevated arterial pressure caused by the contraction of pathological small artery spasm. ACE inhibitors (captopril, enalapril, lisinopril, etc.) are the current medicine for the treatment of hypertension. Although ACE inhibitors can help to regulate blood pressure, but long-term use of these drugs, can cause excessive blood pressure, taste failure, blood vessel elasticity losing, urinary system damage and other side effects.

Hydrophilicity is a major feature of konjac glucomannan. This make it extremely viscous after the absorption of water. Konjac polysaccharides is one of the most viscous polysaccharides currently used in industry. However, its utilization rate is restricted because of its high viscosity (Alon-Sande et al., 2009).

In order to broaden the application range of konjac, the primary method is to degrade konjac polysaccharides into smaller molecular weight oligosaccharides through various means (Xu et al., 2007).

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Table 1
Main compositions of konjac.

Basic ingredient	soluble sugar	reducing sugar	soluble protein
Content (%)	39.28 ± 0.27	5.08 ± 0.45	1.96 ± 0.13

Degraded Konjac oligosaccharides are now widely used in food, medical, chemical, textile, cosmetic, petroleum and other fields. However, these degraded oligosaccharides are mainly a mixture of oligosaccharides with even molecular weight. With low molecular weight they are difficult to be purified by traditional purification methods such as G-serials Sephadex column, therefore, till now no study has been done about its purification and the chemical characteristics of purified product. This greatly limited its industrial application.

The aim of this study was therefore to find out a novel method to purify a konjac oligosaccharide obtained from degradation of konjac polysaccharide and make structure identification of it. In addition, to study its inhibition effect on ACE activity in the hope of developing as an ideal drug for the treatment of ACE.

2. Materials and methods

2.1. Plants materials and reagents

Konjac obtained from Yunnan, China and stored in the Key Laboratory of Food Nutrition and Safety (Ministry of Education, China), College of Food Science and Biotechnology (Tianjin University of Science and Technology, Tianjin, China). D-arabinose and Sephadex G-15 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in this paper were of analytical grade.

2.2. Main ingredient analysis of konjac

The soluble sugar content, reducing sugar content and soluble protein content of the crud polysaccharides were determined, respectively. Soluble sugar content was determined by the phenol-sulfuric acid colorimetric method using glucose as the standard (Dubois et al., 1956). The reducing sugar content (Bappa et al., 2018) was determined by the 3,5-dinitrosalicylic acid colorimetry (DNS) method using glucose as the standard. Soluble protein content was measured by the Bradford method using bovineserum albumin as the standard (Bradford, 1976).

2.3. Extraction procedure of crude polysaccharides from konjac

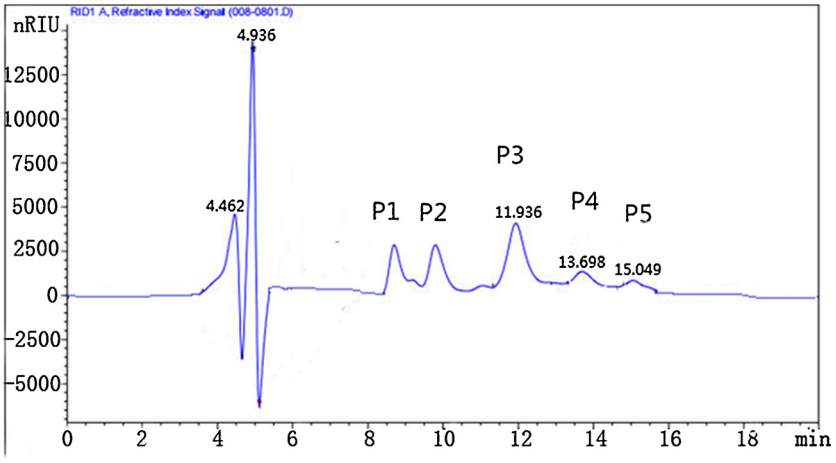

Fig. 1. HPLC chromatogram of KPD.

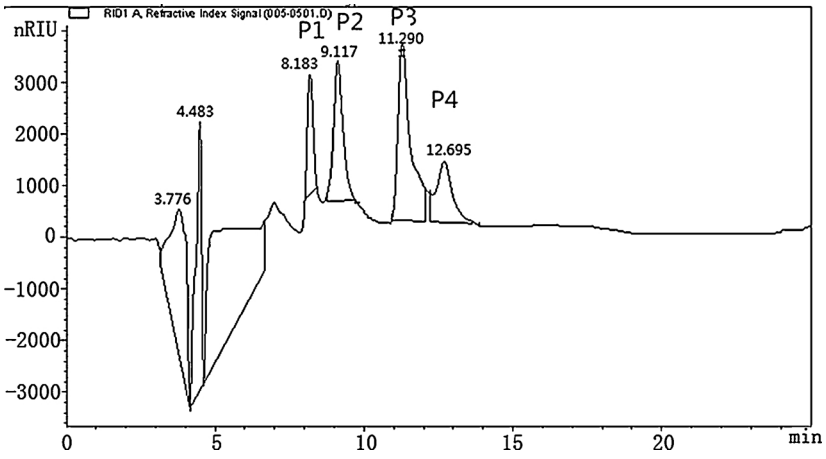

Fig. 2. HPLC chromatogram after KPD alcohol precipitation.