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Physicochemical properties of *Tremella fuciformis* polysaccharide and its interactions with myofibrillar protein

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ABSTRACT

Tremella fuciformis, belonging to the *tremellaceae*, was known as an edible and medicinal fungus in China. A polysaccharide fraction (TPS) in *Tremella fuciformis* was extracted by hot water and its physicochemical as well as rheological properties were investigated in the study. Results showed that TPS contained 0.72% (w/w) uronic acid, 0.60% (w/w) protein and 92.17% (w/w) total sugar, while consisted of rhamnose, xylose, mannose and glucose in the molar ratio of 1.13: 1: 4.70: 0.81. Results of rheological test demonstrated that TPS exhibited properties of pseudoplastic fluids in distilled water and could form weak gel in high frequency at 25°C. Meanwhile, it was found that TPS could significantly enhance the gel hardness and water-holding capacity (WHC) of myofibrillar protein gels. Besides, elasticity of myofibrillar protein gels was improved considerably by adding TPS. IR spectrum analysis of myofibrillar protein-TPS gels indicated that TPS decreased the wave number of N-H and O-H stretching peak between 3000 and 3500 cm^{-1}, suggesting that TPS could enhance hydrogen bonding in myofibrillar protein gels. This study suggests that TPS may have an extensive prospect in the processing of low-fat meat products.

1. Introduction

Nowadays, low-fat foods are increasingly popular in food consumption. However, decreasing the amount of fat in meat would lead to a drop in the quality, such as problems with texture, flavor and mouthfeel (Keeton, 1994). Polysaccharides are now considered as the most effective fat substitutes in the low-fat meat products manufacturing, because they can improve the gelling, thickening, emulsifying and foaming abilities of proteins (Schmitt & Turgeon, 2011). The interactions between polysaccharides and proteins are affected by a number of factors, such as covalent bonds, electrostatic forces, hydrogen bonding, van der Waals force, hydrophobic interaction, ionic bonds and molecular entanglement (De Jong & De Velde, 2007). Although protein and polysaccharide mixtures can form gel at low temperature, the gel-formation mostly occurs in the heating process (Alting, Hamer, De Kruif, & Visschers, 2000). Polysaccharides, due to its gelling characteristic and water retaining property, are promising substitute for fat (Luruenamartinez, Vivarquintana, & Revilla, 2004). Therefore, understanding the interactions between polysaccharide and myofibrillar protein is an essential step in the development of new meat products with enhanced property and lower fat. In fact, many papers have sought to investigate the interaction between polysaccharide and protein (Bernal, Smajda, Smith, & Stanley, 1987). For their ability to retain water, several polysaccharides have also already been used in food industry, such as locust bean gum (Schorsch, Jones, & Norton, 1999), carrageenan (Drohan, Tziboula, Mcnulty, & Horne, 1997), chitosan (Hoven, Tangpasuthadol, Angkitpaiboon, Vallapa, & Kiatkamjornwong, 2007) and k-carrageenan (Cao et al., 2015; Mao et al., 2013), providing a desirable texture and enhance gelling character to protein. It was found that the effects of polysaccharide on the protein depend on multiple parameters, including physical condition, composition, distribution and different volume rate (Filipi & Lee, 1998; Montero, Hurtado, & Perezmateos, 2000). The nonspecific effects of protein and polysaccharide could induce the formation of compound or incompatibility polymer (Ramirez, Barrera, Morales, & Vazquez, 2002).

Tremella fuciformis (Fig. 1A) is a kind of fungus that has high nutritional and medicinal values in China. The polysaccharides, obtained from *Tremella fuciformis* fruit body, have been regarded as the major active component related to the nutritional and health of *Tremella fuciformis* (X. Wang, Z. Zhang & M. Zhao, 2015). It has been well known that *Tremella fuciformis* polysaccharides (TPS) (Fig. 1B) can be classified into five categories: acidic polysaccharide (Ukai, Kiho, & Hirose, 1972), neutral polysaccharide (Ukai, Kiho, Hara, & Hirose, 1978), acidic

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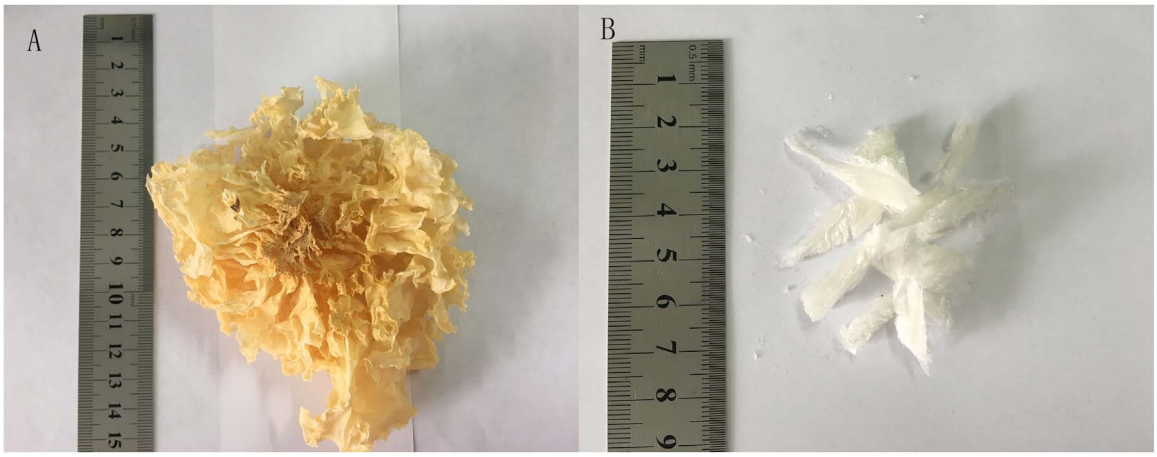

Fig. 1. Pictures of *Tremella fuciformis* (A) and the TPS (B).

oligosaccharide (Ukai et al., 1972), cell wall polysaccharide (Sone & Misaki, 1978) and exopolysaccharide (Kakuta, Sone, Umeda, & Misaki, 1979), While mannose was the main active ingredients in *Tremella fuciformis* polysaccharide (Laemmli, 1970). Although the structure (Kiho et al., 2000), conformation (Liu et al., 2016) and bioactivity (X. Wang et al., 2015) of TPS have been extensively studied, the knowledge of the dynamic rheology of TPS in aqueous solution is still very limited.

The rheological properties of polysaccharide solution are very sensitive to the molecular structure (Chamberlain & Rao, 2000). Research on structure-activity relationship of active polysaccharide demonstrated that the viscosity of the polysaccharide has great influence on its active function in the body (Dickinson, 2011; Schmitt, Sanchez, Desobrybanon & Hardy, 2010; Schmitt & Turgeon, 2011). The rheological study of polysaccharides not only has important significance in sciences, but also in food, medicine, cosmetics and other related industries (Zhang, Xu, Xu, & Zhang, 2007).

In this paper, we investigated the chemical composition and rheological properties of a polysaccharide fraction (TPS) isolated from *Tremella fuciformis* and its interaction with myofibrillar protein, hoping to provide a theoretical foundation for the application of TPS in food industry.

2. Materials and methods

2.1. Materials

Tremella fuciformis and pork were obtained from a local supermarket of Carrefour Group in Hefei, China. Standard monosaccharides (D-xylose, D-glucose, D-galactose, L-rhamnose, L-arabinose, D-mannose) were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). All the reagents were of analytical grade.

2.2. Extraction of TPS

TPS was extracted according to previous method with some modifications (Xie et al., 2014). Dried *Tremella fuciformis* (Fig. 1A) was processed to fine powder by a blender. Subsequently, starch in *Tremella fuciformis* was removed by using α-amylase according to a previous paper and the powders were defatted with petroleum ether for 12 h (Agbenorhevi, Kontogiorgos, Kirby, Morris, & Tosh, 2011). The residues were extracted twice with distilled water at 100 °C for 2 h. The supernatants were filtered with four layers of gauze, then four times volume of 95% ethanol was added into the supernatant for precipitation for 24 h at 4 °C. The supernatants were removed by centrifugation at 10,000g for 10 min. The precipitation was dissolved in water and deproteinized by Sevage method. Then the solution was dialyzed, and lyophilized to obtain the polysaccharide fraction TPS (Fig. 1B).

2.3. Physicochemical characterization of TPS

2.3.1. Determination of total sugar, protein and uronic acid contents

The total sugar content of TPS was determined using phenol-sulfuric acid method. In short, 1 mL of 5% phenol aqueous solution and 5 mL of concentrated sulfuric acid were added into 1 mL 0.1 mg/mL TPS solution. The mixed solution was placed at room temperature for 30 min. After that, the absorbance value of the mixture was assayed at 485 nm.

The protein content was measured using Coomassie brilliant blue method and the bovine serum albumin was used as standard (Lowry, Rosebrough, Farr, & Randall, 1951).

Uronic acid was determined using the method of m-hydroxybiphenyl and the galacturonic acid was used as standard (Blumenkrantz & Asboe-Hansen, 1973).

2.3.2. UV spectroscopy analysis

The Ultraviolet spectrum of TPS solution at 5.0 mg/mL was scanned on a UV–vis spectrophotometer in the range of 200–400 nm (Yang et al., 2009).

2.3.3. Monosaccharide composition analysis

The monosaccharide composition analysis of TPS was performed by gas chromatography (GC) (Wang, Luo, Zha, & Feng, 2010; Xie et al., 2016). 10 mg TPS was hydrolysed with 4 mL of 2 M trifluoroacetic acid (TFA) in a sealed glass tube for 4 h at 120 °C. The residual TFA was removed by rotary vacuum evaporator under reduced pressure. Then 30 mg of $NaBH_4$ were reacted with the monosaccharides for 3 h under ambient temperature after dissolved in distilled water. 25% acetic acid was used to neutralize the reaction solution until no air bubble generation. When the aqueous phase was removed after reaction, the residue sample was reacted with 3 mL of pyridine and 3 mL of acetic anhydride for 1 h at 100 °C. GC was used to analysis the reaction products.

2.4. Rheological properties of TPS solutions

2.4.1. Sample preparation

TPS was dissolved in distilled water at the concentrations of 14, 16, 18, 20, 22, 24 mg/mL, respectively.

2.4.2. Detection of linear viscoelastic region

The changes of storage modulus (G') and loss modulus (G'') as a function of strain were measured by a rheometer (DHR-3, TA, USA) equipped with a parallel plate geometry of 40 mm diameter. The measurement was performed with the angular frequency of 6.28 rad/s