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Antioxidant activities of polysaccharides obtained from *Chlorella pyrenoidosa* via different ethanol concentrations

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ABSTRACT

An ultrasonic-assisted extraction of *Chlorella pyrenoidosa* polysaccharides (CPP) was carried out using different concentrations of ethanol for precipitation, and named as CPP60, CPP70 and CPP85, respectively. The monosaccharide composition of each polysaccharide (CPP) was determined using gas chromatography (GC) and the antioxidant activity of each was investigated via the reducing power and scavenging activity of hydroxyl radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and superoxide anion radicals, respectively. All of the polysaccharides examined possessed antioxidant activity *in vitro*. CPP70 exhibited stronger scavenging activity against superoxide, DPPH and hydroxyl radicals, when compared with CPP60 and CPP85. This suggests that polysaccharides from *C. pyrenoidosa* precipitated by a final ethanol concentration of 70%, have the potential to be developed as natural antioxidants for use in food and pharmaceuticals.

1. Introduction

Recently, microalgae have received quite a lot of attention as potential commercial food supplements given that they are rich in various bioactive compounds, such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins and sterols [1]. Most of these bioactive compounds exhibit anti-inflammatory, antimicrobial, immunological, and antiviral activity and can protect against toxic reactive oxygen species (ROS) [2–5].

Chlorella is a unicellular green microalgae, which exists in both fresh and marine water [6]. The chlorella growth factor (CGF), extracted by water from chlorella cells, has the ability to promote tissue regeneration, cell growth and division, formation of antibody producing lymphocytes, resistance against gamma irradiation and tumors, and enhancement of immunity [7]. CGF contains free amino acids, peptides, glycoproteins, polyamines, phytohormones, vitamins, minerals, and other unknown components [8]. Thus, Chlorella has been widely used as a supplement for human nutrition and in animal feed. Water extracts from Chlorella are complicated, thus it is difficult to distinguish which compounds are the most bioactive; however recent research indicates that the bioactivity might be associated with polysaccharides [9].

The Food and Agriculture Organization of the United Nations (FAO) has named *C. pyrenoidosa* a green healthy food [10]. Most research into the bioactivity of polysaccharides extracted from *C. pyrenoidosa* has focused on antitumor and immune activities [11,12]; however a few studies have concentrated on antioxidant activity. Furthermore, to the best of our knowledge, there have been no published studies on the antioxidant bioactivities of *C. pyrenoidosa* polysaccharides precipitated with different ethanol concentrations. Traditionally, ethanol has been used for the initial purification of an aqueous extract, since it is simple, rapid and easily scalable [13]. Importantly, ethanol concentration is a decisive factor in the structural features and molecular size of polysaccharides, which may be related to polysaccharide bioactivity [14]. However, few studies have taken ethanol concentration into account when preparing polysaccharide samples. In the current study, the chemical composition and antioxidant activity of polysaccharides from *C. pyrenoidosa* were estimated after precipitation using different concentrations of ethanol. In addition, the antioxidant capabilities of three differently-precipitated polysaccharide fractions were investigated with the aim of finding a new natural antioxidant for human health.

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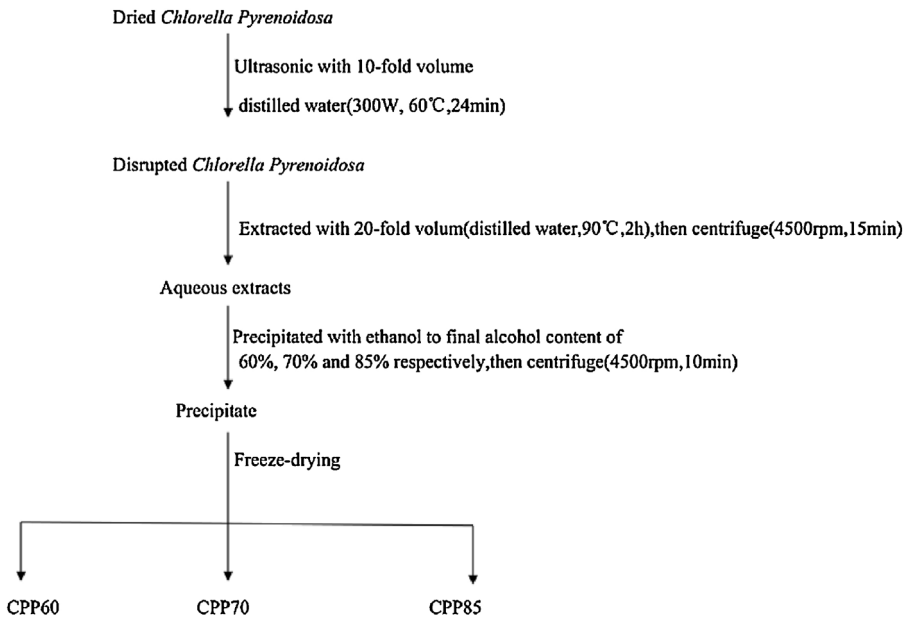

Fig. 1. Extraction process and different polysaccharide fractions from *C. pyrenoidosa.*

2. Materials

The *C. pyrenoidosa* was obtained from Freshwater Algae Culture Collection at the Institute of Hydrobiology (Wu Han, China). Rhamnose, arabinose, xylose, mannose, glucose, galactose and fructose were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). All other chemicals were of analytical grade.

3. Methods

3.1. Preparation of the polysaccharide ***extract***

C. pyrenoidosa has a rigid cell wall, which is comprised mainly of cellulose and thickens during maturation [15]. Therefore, it is necessary to disrupt the cell wall prior to extraction. The *C. pyrenoidosa* sample was treated in an ultrasonic processor at 300 W, with 10-fold distilled water, for 24 min. The ratio of solid to liquid was adjusted to 1:30 (W/V). The extraction process was carried out twice with distilled water. The residue was separated by centrifugation at 4500 rpm for 15 min, and the supernatant was concentrated to one-fifth of the original volume using a rotary evaporator at 55 °C under vacuum. Appropriate volumes of ethanol were added to the concentrated solution to obtain final ethanol concentrations of 60% (CPP60), 70% (CPP70), and 85% (CPP85), respectively. The mixture was stored overnight at 4 °C, and then centrifuged at 4500 rpm for 10 min to obtain the precipitates. After precipitates were freeze dried, the CPP60, CPP70 and CPP85 were obtained (Fig. 1).

3.2. Chemical analysis

The chemical composition analysis of precipitates prepared above was conducted, including total sugar, protein and total phenol contents. Total sugar contents were determined by phenol-sulfuric acid method using D-glucose as standard [16], protein contents were determined by Coomassie brilliant blue method using vine serum albumin as standard [17], and total phenol contents were assayed by FoLin-CicoaLteu's regent method and using gallic acid as standard [18].

3.3. Monosaccharide composition analysis

The monosaccharide composition analysis of the precipitates (CPP60, CPP70 and CPP85) was determined by Gas Chromatography (GC) according to the method of Pu et al. [19] with some modifications. In brief, the sample was hydrolyed with 2 ml 2M triflouroacetic acid (TFA) at 110 °C in a sealed-tube for 4 h. The TFA was removed and cooled down to room temperature, thereafter 10 mg of hydroxylamine hydrochloride and 0.5 mL of pyridine were added and incubated at 90 °C for 30 min. A 0.5 mL of acetic anhydride was added into mixture to continue reacting for 30 min at 90 °C to achieve glyc-nitrile derivatization. GC analysis was performed by an instrument (Agilent 7890A, Agilent Technologies Co. Ltd.,USA) equipped with an DB − 1701 quartz capillary column (30 m × 0.32 mm, 0.25 μm) and a flame-ionization detector (FID). The initial temperature was set at 170 °C.and maintained for 2 min, then increased to 250 °C at the rate of 10 °C/min and kept at 250 °C for 30 min. The detector and injector temperatures was 250 °C. L-rhamnose, D-arabinose, D-xylose, D-mannose, D-glucose, D-galactose and D-fructose were used as standards and the molar ratio of monosaccharide in the polysaccharide samples was calculated.

3.4. Antioxidant activity assays

The polysaccharides (CPP60, CPP70 and CPP85) prepared above was diluted with distilled water into different concentrations of 0.4 mg/mL, 0.8 mg/mL, 1.2 mg/mL, 1.6 mg/mL and 2 mg/mL. The antioxidant activity was investigated by reducing power, scavenging activity of hydroxyl, DPPH and superoxide anion radicals.

3.4.1. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method of Liu et al. [20] with some modifications. Briefly, 1 mL of $FeSO_4$ (9 mM) and 1 mL of salicylic acid–ethanol (9 mM) were added to 1 mL of polysaccharide sample, 1 mL of H_2O_2 (9 mM) was added finally to startthe reactions, then incubated at