Carbohydrate Polymers 111 (2014) 584–587

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Short communication

Extraction, purification and antioxidant activities of the polysaccharides from maca (*Lepidium meyenii*)

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ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 11 May 2014
Accepted 13 May 2014
Available online 21 May 2014

Keywords:
Maca
Lepidium meyenii
Polysaccharides
Monosaccharide composition
Antioxidant activity

ABSTRACT

Water-soluble polysaccharides were separated from maca (*Lepidium meyenii*) aqueous extract (MAE). The crude polysaccharides were deproteinized by Sevag method. During the preparation process of maca polysaccharides, amylase and glucoamylase effectively removed starch in maca polysaccharides. Four *Lepidium meyenii* polysaccharides (LMPs) were obtained by changing the concentration of ethanol in the process of polysaccharide precipitation. All of the LMPs were composed of rhamnose, arabinose, glucose and galactose. Antioxidant activity tests revealed that LMP-60 showed good capability of scavenging hydroxyl free radical and superoxide radical at 2.0 mg/mL, the scavenging rate was 52.9% and 85.8%, respectively. Therefore, the results showed that maca polysaccharides had a high antioxidant activity and could be explored as the source of bioactive compounds.

1. Introduction

Maca (*Lepidium meyenii*) is a native plant in the Andes region and belongs to the Brassicaceae family. It is grown in altitudes varying between 3700 and 4450 m (León, 1964). Maca root has been used for centuries in the Andes to enhance fertility in humans and animals (Flores, Walker, Guimaraes, Bais, & Vivanco, 2003). The maca root contains high nutritional value component, such as protein (10–18%), carbohydrates (59–76%), as well as a high number of free amino acids and considerable mineral contents (Dini, Migliuolo, Rastrelli, Saturnino, & Schettino, 1994). The biological activity of maca includes energizer (Stone, Ibarra, Roller, Zangara, & Stevenson, 2009), fertility-enhancer (Ruiz-Luna et al., 2005) properties, improving memory and learning (Cordova-Ruiz, 2011). However, compared with the numerous studies of maca biological activity, little attention was devoted to the extraction and investigation of maca (*Lepidium meyenii*) polysaccharides (LMP).

In the present study, polysaccharides were extracted from maca, the purification condition, the effect of reagent on the polysaccharide precipitation, monosaccharide composition and the antioxidant activities of LMPs were investigated.

2. Materials and method

2.1. Materials

The roots of *Lepidium meyenii* were obtained from Yunnan province, China. The materials were air-dried at room temperature. Other reagents were of analytical grade as commercially available.

2.2. Polysaccharide extraction and treatment

Lepidium meyenii powder (40 mesh) was extracted with hot water (80 °C) for 1 h. The solution was centrifuged at 5000 rpm for 30 min, then the supernatant was concentrated by rotary vacuum evaporator at 60 °C, and subsequently was dried with a spray dryer. A certain amount of aqueous extract of maca was dissolved in 200 mL water, and 2 mL 10% amylase and 1 mL 0.2 M phosphate buffer (pH 6.5) was added then. After enzymatic hydrolysis in water bath at 50–60 °C for 2 h, 1 mL glucoamylase was added and incubated for 1 h. Enzymatic solution was heated to 100 °C rapidly to inactivate enzyme reaction, and then cooled and

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http://dx.doi.org/10.1016/j.carbpol.2014.05.017
0144-8617/

Table 1
Effect of enzymatic pre-treatment, filtration and removal of protein on the yield and purity of LMP.

No.	Enzyme treatment	Filtration	Centrifuge	Deproteinization	Polysaccharide yield (%)	Polysaccharide purity (%)
1	Y	N	Y	N	11.8	48.4
2	Y	N	Y	Y	10.8	50.8
3	N	N	Y	N	17.8	69.7
4	N	N	Y	Y	17.1	64.2
5	N	Y	N	N	15.7	81.4
6	N	Y	N	Y	15.5	71.0

Enzyme treatment: amylase and glucoamylase; filtration: the solution was filtered with filter paper; centrifuge: the solution was centrifuged with 4000 r/min; deproteinization: Sevag method was used to remove protein; Y: treatment; N: no treatment.

diluted to 250 mL. The diluents extract solution was centrifuged at 4000 rpm for 30 min, then the supernatant was deproteinized by using Sevag's method. The deproteinized supernatant was then precipitated at final ethanol concentration of 60%, 70%, 80% and 90% to get polysaccharides named LMP-60, LMP-70, LMP-80 and LMP-90, respectively. After centrifugation, the precipitate was washed with anhydrous ethanol, acetone and ether in turn, and then dried to yield the polysaccharides (LMP).

$$\text{Polysaccharides yield (\%)} = \frac{\text{Polysaccharides weight}}{\text{Raw material weight}} \times 100\% \quad (1)$$

Polysaccharide purity was measured using the phenol–sulfuric acid method. Protein content in LMPs was determined by Bradford method. FT-IR of polysaccharides was carried out on Fourier transform-infrared spectrometer in the range of 500–4000 cm^{-1}.

Monosaccharide composition was determined as follows: polysaccharide (20 mg) was dissolved in 4 mL 2 mol/L trifluoroacetic acid solution (TFA) and hydrolyzed at 110 °C for 2 h. The resulting solution was concentrated under reduced pressure and the excess of acid was removed by repeated co-distillations with methanol. The monosaccharides were analyzed on a waters NH_2 (4.6 mm × 250 mm, 5 μm) column kept at 40 °C with acetonitrile–water (85:15) as the mobile phase at a flow rate of 1.0 mL/min, and the injection volume was 10 μL.

The hydroxyl radical, superoxide radicals and DPPH scavenging activity of samples were measured according to the method of Yao et al. (2012).

3. Results and discussion

3.1. Characteristics of LMPs

The effects of enzymatic pre-treatment, filtration and removal of proteins on the yield and purity of LMPs are shown in Table 1. It can be seen from the results, under the same conditions, experiment 1 compares with experiment 3, and experiment 2 compares with experiment 4, the yields of polysaccharides without enzymatic hydrolysis are higher than those with enzymatic hydrolysis by 6%. The reason for this may be that raw materials contained a certain amount starch, starch was also precipitated with polysaccharides.

In order to investigate the effect of the order of enzymatic hydrolysis on polysaccharides purity, experiment 1 was changed as follows: the solution was centrifuged firstly, and then precipitated with 80% ethanol, polysaccharide was hydrolysed before a further precipitation with 80% ethanol. The resulting polysaccharide yield and purity were consistent with those of the original experiment 1. The results showed that the order of enzymatic hydrolysis had no effect on polysaccharides yield and purity.

According to the above results, LPMs were prepared by ethanol precipitation, followed by enzymolysis, centrifugation and deproteinization. The yield and purity of LMPs are shown in Table 2. Clearly, the increase in ethanol concentration from 60% to 90% resulted in the increase of yield from 5.2% to 15.0%, but the purity declined from 69.4% to 39.5%. The reason may be that, the higher ethanol concentration, i.e. the less polarity, the more beneficial to polysaccharide precipitation. The reason for the lower purity may be that non-polysaccharide substances were precipitated

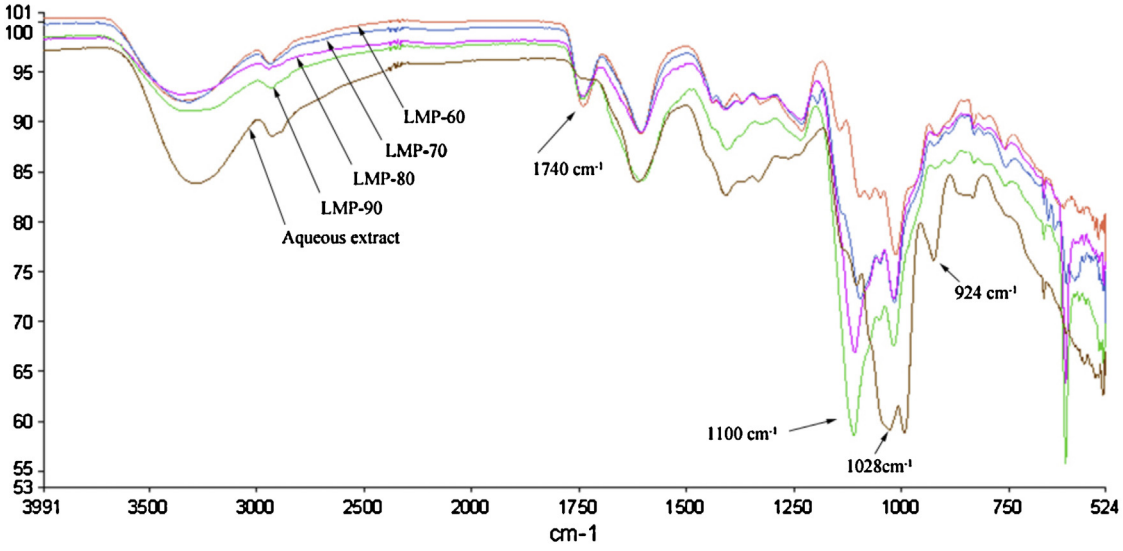

Fig. 1. IR spectrum of the LMPs and maca aqueous extract.