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Short communication

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

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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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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

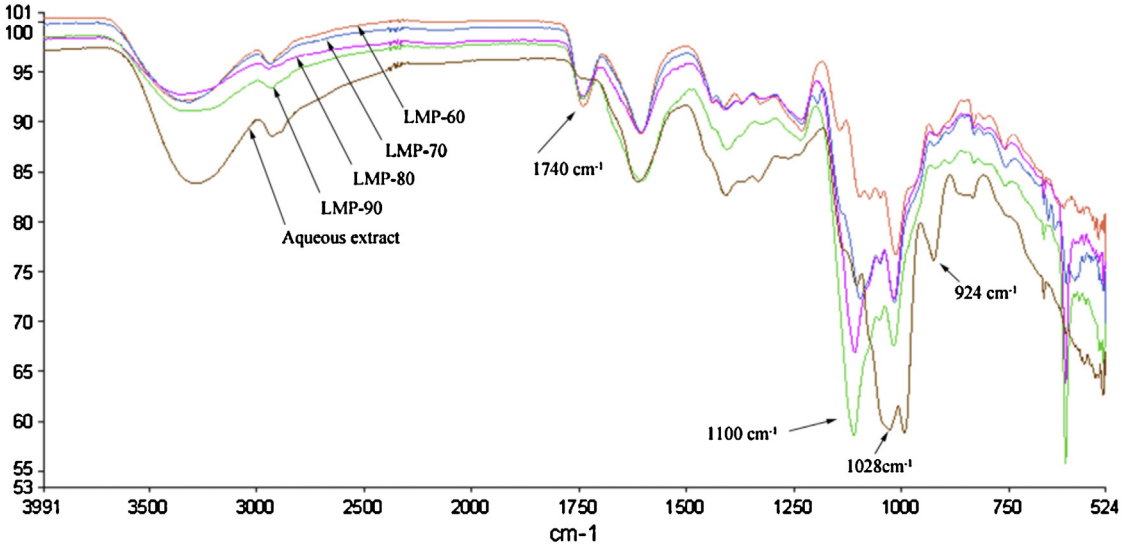

Fig. 1. IR spectrum of the LMPs and maca aqueous extract.

Table 2
The yield and purity of LMPs.

Samples	Ethanol concentration (%)	Polysaccharide yield (%)	Polysaccharide purity (%)
LMP-60	60	5.2	69.4
LMP-70	70	8.0	59.0
LMP-80	80	10.8	50.8
LMP-90	90	15.0	39.5

Table 3
The yield and purity of polysaccharide precipitated by isopropanol.

Samples	Isopropanol concentration (%)	Polysaccharide yield (%)	Polysaccharide purity (%)
1	40	1.15	70.2
2	50	3.67	65.4
3	60	6.17↑	55.9↓
4	70	10.5↑	53.0↓
5	80	16.6↑	43.5↓
6	90	25.7↑	41.6↑

Table 4
Monosaccharide compositions of LMPs.

Samples	Rhamnose	Arabinose	Glucose	Galactose
LMP-60	1.81	6.85	1	3.21
LMP-70	2.18	9.47	1	5.21
LMP-80	1.49	6.87	1	3.5
LMP-90	0.83	2.68	1	1.32

together with polysaccharides in pace with increasing ethanol concentration. Firstly, with the ethanol concentration increased, other components were also precipitated. Secondly, the higher the ethanol concentration, the harder to spread the polysaccharide, resulting in the difficulty of purifying polysaccharides.

In order to verify the effects of polarity of precipitation reagent on the yield and purity of polysaccharides, instead of ethanol, isopropanol was used to precipitate the polysaccharide (see Section 2.2). The results are shown in Table 3. Compared to ethanol precipitation, isopropanol precipitation of polysaccharide was better to get more polysaccharides. Clearly, an increase in isopropanol concentration from 40% to 90% resulted in the increase of yield from 1.15% to 25.7%, but the purity declined from 70.2% to 41.6%. To achieve the same precipitation effect, the amount of isopropanol was less than that of ethanol. The reason was that the polarity of isopropanol is smaller than ethanol. As the concentration of isopropanol increased, precipitated polysaccharide increased. Results had the same trend which was consistent with ethanol precipitation. The results proved that the more smaller the polarity, the more beneficial to precipitate polysaccharide. However, due to the micro-toxicity of isopropanol, the method of ethanol precipitation is still used for preparing maca polysaccharides.

The monosaccharide compositions of LMPs are shown in Table 4. The results indicated that arabinose was the major monosaccharide construction of LMPs. The molar ratio of monosaccharide compositions in LMP-60 was described as follows: rhamnose:arabinose:glucose:galactose = 1.81:6.85:1:3.21. Monosaccharide proportion of LMP-70 was rhamnose:arabinose:glucose:galactose = 2.18:9.47:1:5.21. Monosaccharide proportion of LMP-80 was rhamnose:arabinose:glucose:galactose = 1.49:6.87:1:3.5. LMP-90 was also composed of four monosaccharides: rhamnose:arabinose:glucose:galactose = 0.83:2.68:1:1.32.

FT-IR spectra of LMPs and maca aqueous extract are shown in Fig. 1. Fig. 1 shows that the spectra of LMPs are typical polysaccharide spectra with a strong and wide stretching peak around 3332 cm^{-1} for O—H stretching vibrations and a weak absorption peak of about 2934 cm^{-1} for C—H stretching

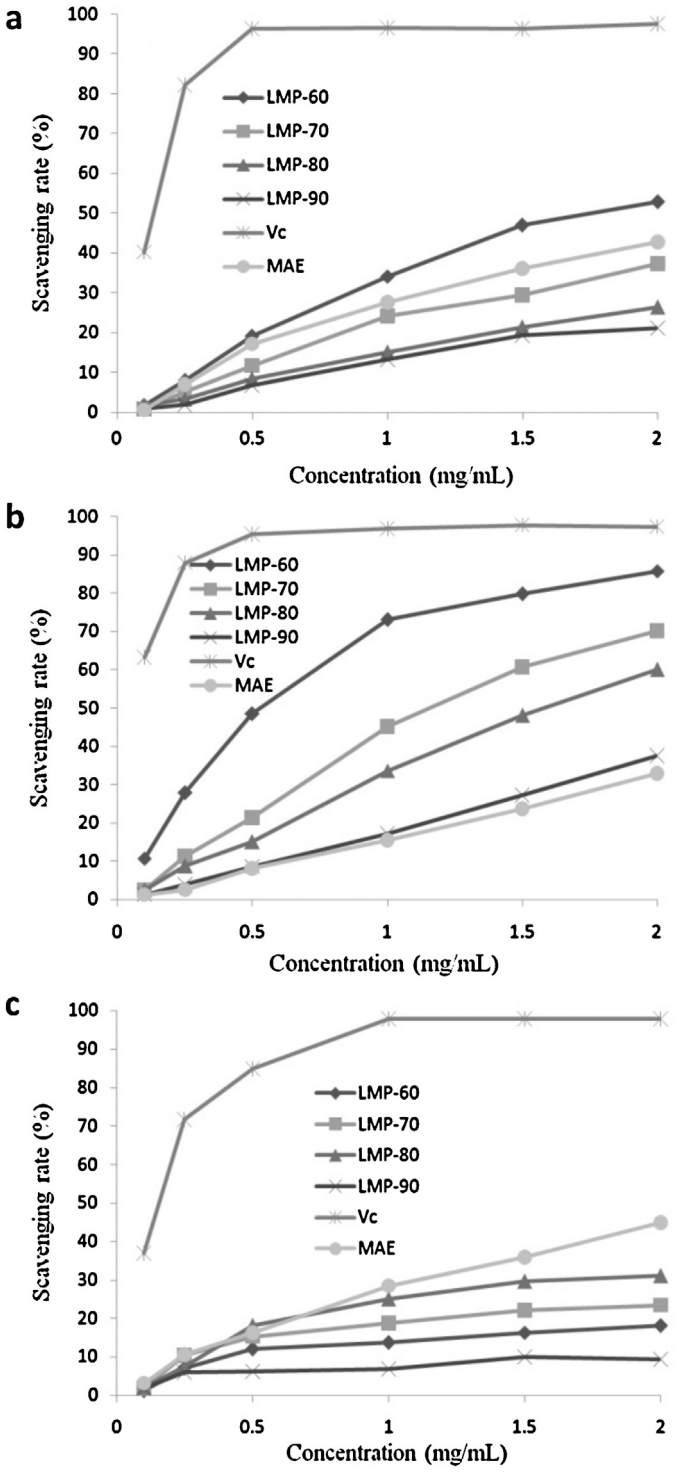

Fig. 2. Antioxidant activities of LMPs, MAE (maca aqueous extract) and Vc. (a) Hydroxyl radical. (b) Superoxide radical. (c) DPPH radical.

vibrations, respectively. Absorptions of 1740 cm^{-1} and 1610 cm^{-1} are attributed to carbonyl group bending vibration and 1237 cm^{-1} and 761 cm^{-1} are C—O—C vibration. In the spectra of LMP-80 and LMP-90, the strong absorption of 1100 cm^{-1} is the characteristic peaks of primary alcohol, but in LMP-60, LMP-70, this peak disappears. In the spectra of maca aqueous extract, 1740 cm^{-1} and 1100 cm^{-1} peaks disappear, instead of two new peaks 1028 cm^{-1} and 924 appeared. This means the maca aqueous extract contains lipids and proteins except for polysaccharides.

3.2. In vitro antioxidant activities

Antioxidant activities of LMPs were tested and the results are presented in Fig. 2. The scavenging ability of the four polysaccharide types on hydroxyl radical and superoxide radical exhibited in a concentration-dependent manner significantly (Fig. 2a and b). Among all samples, the scavenging ability on hydroxyl radical decreased in the order: Vc > LMP-60 > MAE > LMP-70 > LMP-80 > LMP-90. The highest hydroxyl radical scavenging ability of 52.9% was obtained by LMP-60 at the concentration of 2.0 mg/mL.

LMPs showed good capability of scavenging superoxide radical. At the concentration of 2.0 mg/mL, the scavenging rate of LMP-60 was 85.8% (Fig. 2b). The order of the superoxide radical scavenging ability of LMPs were Vc > LMP-60 > LMP-70 > LMP-80 > LMP-90 > MAE.

The DPPH radical scavenging ability of LMPs was lower than that of hydroxyl radical and superoxide radical. Maca aqueous extract exhibited higher DPPH radical scavenging activity than LMPs (Fig. 2c). The order of the DPPH radical scavenging ability of LMPs were Vc > MAE > LMP-60 > LMP-70 > LMP-80 > LMP-90. At 2 mg/mL, the scavenging rate of MAE and LMP-80 was 44.8% and 29.5%. The reason may be that some antioxidants, especially the anti-DPPH substances, were removed in the process of ethanol precipitation polysaccharide.

From the above results, maca polysaccharides exhibited potential antioxidant capacities and could be explored as a source of bioactive compounds.

4. Conclusions

Water-soluble polysaccharides were separated from maca aqueous extract. Maca aqueous extract was treated with amylase and glucoamylase to remove starch. Maca polysaccharides were obtained by ethanol precipitation method. All of the polysaccharides 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. In addition, maca aqueous extract exhibited higher DPPH radical scavenging activity than LMPs.

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