Multimode ultrasonic extraction of polysaccharides from maca (Lepidium meyenii): Optimization, purification, and in vitro immunoregulatory activity

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ABSTRACT

This study evaluates the effect of multimodal ultrasound on the extraction efficiency and immunoregulatory activity of polysaccharides from Lepidium meyenii Walp. (LMP). The separation and purification of maca polysaccharides were investigated by the DEAE-52 cellulose column, and the monosaccharide compositions were identified by HPGPC. Their immune activity was analyzed by the secretion of cytokines (TNF-α and IL-6) from RAW 264.7 macrophage. The results showed that the optimal extraction conditions were energy aggregation alternating dual-frequency ultrasound (EADU) with frequency combinations of 20/35, extraction time of 15 min, material/water ratio of 1:10 g/mL, ultrasonic power intensity of 150 W/L, intermittent time ratio of 4 s/3 s, and extraction temperature of 50 °C. The extraction rates of purified polysaccharides (US3) increased by 44.90%. The LMP extracted by EADU contained arabinose, galactose, and glucose in the molar ratios of 2.9:2.72:5.05. In addition, US3 promoted the release of TNF-α and IL-6 from RAW 264.7 better than RS3 (purified polysaccharides extracted by hot water), which indicated that US3 exerted remarkable immune activity. It could be an excellent functional additive in food or medicine.

1. Introduction

Maca (Lepidium meyenii Walp.) belongs to the Brassicaceae family with tubers shaped like radishes and mainly grows in the Peruvian Andean region [1]. Maca has been cultivated for more than 2000 years because of its multiple nutritional and medicinal values, especially its root, which consists of protein (10–18%), carbohydrates (59–76%), various free amino acids, trace elements, and considerable minerals [2]. Traditionally, it was always used to enhance fertility, energy, and physical strength [3]. In recent years, various biological components extracted from maca, including polysaccharides, alkaloids, and polyphenols, have attracted more attention from researchers [4]. Maca polysaccharides (LMP), as one of the main ingredients, have garnered particular focus. It has been proven that maca polysaccharides have various biological properties, including anti-fatigue activity [5], prevention of alcohol-induced liver injury [6], antioxidant activity [7], and memory impairment [8], immunoregulatory activities [3], etc.

The traditional method for extracting polysaccharides is hot water, but it is time-consuming, inefficient, and has a low extraction rate [9]. Recently, to overcome the disadvantages of traditional extraction, novel extraction technologies have been developed, such as microwave, supercritical fluid, enzymatic, and ultrasonic-assisted extraction [10]. Ultrasonic has been widely concerned with green, safe, and efficient technology. Ultrasonic-assisted extraction could improve the extraction yield and shorten the extraction time. At the same time, it could show good biological activity. It is generally considered that the mechanical effect, caviation effect, and thermal effect significantly influence the ultrasonic extraction process [11]. These comprehensive effects could destroy the cell walls, increase the penetration of the solvent, and accelerate the transformation of active ingredients in the cell to the solvent, which enhances the diffusivity, solubility, and transport of solute molecules [12]. However, many researchers study a single ultrasonic device without systematically looking at various ultrasonic modes, making it challenging to obtain the optimal processing conditions. Therefore, it is of great significance to screen different ultrasonic frequencies and working modes to maximize the extraction rate of active ingredients.

It has been reported that botanical polysaccharides are ideal immunomodulatory agents due to their non-cytotoxic properties [13]. Macrophages are considered to be important target cells for some anti-tumor and immunomodulatory agents, which are the first cells to recognize infectious agents [14]. In the immune system, RAW 264.7...
macrophages play a significant role in inducing and regulating specific immune systems owe to their phagocytosis and antigen presentation abilities [15]. Hence, RAW 264.7 macrophages are usually used to evaluate the immunomodulatory activity of substances.

In this study, the optimal ultrasonic working mode was selected based on maca polysaccharide content. Subsequently, the extraction parameters were optimized. The different samples (crude extract, crude polysaccharide, and purified polysaccharide) were applied to RAW 264.7 macrophages. The survival rate and the levels of pro-inflammatory cytokines (TNF-α and IL-6) were determined to evaluate the toxicity and immune activity of maca polysaccharides.

2. Materials and methods

2.1. Materials

Maca was obtained from Qinghai Shennong Biotechnology Co., Ltd., China; RAW 264.7 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China; TNF-α and IL-6 ELISA kits were purchased from Huijia Biological Technology Co., LTD, Xiamen, China. MTT Cell Proliferation and Cytotoxicity Assay Kit and Lipopolysaccharides (LPS) were obtained from Sigma Chemical Co., USA. Other reagents used in the study were analytical grades.

2.2. Extraction process

Before extraction, maca roots were chopped and powered using a pulverizer and filtered through a 60-mesh sieve. 33.3 g of maca powder was extracted with 1 L distilled water at 60 °C using a magnetic stirring water bath. After Ultrasonic assisted extraction, the samples were centrifuged at 4000 rpm for 20 min. The supernatant was concentrated to the thirtieth of the volume with a rotary evaporator. The ultrasonic crude extract (US1) was obtained by freeze-drying. The hot water crude extract (HS1) was obtained without ultrasonic treatments. Four times the volume of ethanol was added to the concentrated sample and kept overnight at 4 °C. After centrifugation, the supernatant was discarded, and the precipitate was freeze-dried to obtain the ultrasonic crude polysaccharide (US2) and hot water crude polysaccharide (RS2). The ultrasonic crude extract and the hot water crude extract were dissolved in water and removed protein by Sevag’s method. The deproteinized supernatant was precipitated by different gradients of ethanol. Afterward, the precipitate was washed twice with acetone and ether, respectively, and then dried to obtain the pure ultrasonic polysaccharide (US3) and hot water pure polysaccharide (RS3).

2.3. Optimization of ultrasonic extraction

2.3.1. Ultrasonic mode and ultrasonic frequency

The samples were sonicated under the following three ultrasonic working modes: energy aggregation counter flow single-frequency ultrasonic (ESU) (28, 35, 40, and 50 kHz); energy aggregation counter flow simultaneous double-frequency ultrasound (EDSU) (20/28, 20/35, 20/40, and 20/50 kHz); energy aggregation counter flow alternation-dual-frequency ultrasound (EADU) (20/28, 20/35, 20/40 and 20/50 kHz); and divergence triple-frequency ultrasound (DTU) (20/28/40, 20/35/50 and 20/40/60 kHz). For the above ultrasonic-assisted treatments, the ultrasonic power intensity was 250 W/L; the solid-liquid ratio was 1:30 g/mL; the extraction temperature was 50 °C; the ultrasonic pulse on-time and off-time was 4 s and 3 s, respectively. During the extraction process (60 min), maca polysaccharides were determined every five minutes in the first half-hour and every ten minutes in the last half hour. The polysaccharides extracted by hot water were set as the control.

2.3.2. Ultrasonic operating parameters

Extraction temperature, ultrasonic intermittent time ratio, a ratio of liquid to material, and ultrasonic power intensity were discussed under the optimized ultrasonic mode and frequency. In detail, maca polysaccharides were extracted with the designed extraction temperature (20, 30, 40, 50 and 60 °C, respectively), ultrasonic intermittent time ratio (2 s/3 s, 4 s/3 s, 6 s/3 s, 4 s/1 s and 4 s/6 s, respectively), solid/liquid ratio (1:10, 1:20, 1:30, 1:40 and 1:50 g/mL, respectively), ultrasonic power intensity (100, 150, 200, 250, 300 W/L, respectively). Each test was conducted in triplicates.

2.4. Determination of maca polysaccharides

The polysaccharides were determined by the sulfuric acid-phenol method with slight modification [16]. Glucose standard solutions with different concentrations were added phenol and sulfuric acid to obtain the standard curve (y = 0.148x + 0.0025, R2 = 0.9992) by measuring the absorbance at 490 nm. The samples were treated in the same way. Polysaccharide contents were calculated by the following equation:

\[ T = \frac{C \times N \times V \times f}{m} \]

Where T was the maca polysaccharide content (%); C was the concentration of polysaccharides (mg/100 mL) according to the standard curve; V was the sample liquid volume; f was the conversion factor (the value was 1.8) calculated by “actual polysaccharide concentration/ measured polysaccharide concentration; m was the sample weight.

The extraction rate of maca polysaccharides was calculated by Eq. (2).

The extraction rate of polysaccharides (%) = \( \frac{m_0}{m} \times 100\% \)

M0 was the polysaccharide weight (g); m was the sample weight.

2.5. Fourier transform infrared analysis of polysaccharide

Fourier transform infrared (FT-IR) studies of US1 and RS1 were determined by the KBr-disk method. The dried polysaccharide was thoroughly mixed with the dried KBr powder, and the tablets were pressed. Subsequently, the tablets were analyzed by a Fourier Infrared Spectrometer (Thermo, Nicolet 380, USA) in the range of 500 to 4000 cm⁻¹.

2.6. Purification of polysaccharides

100 mg of US3 and RS3 were dissolved in 10 mL deionized water, respectively, and centrifuged (10000 rpm/min, 10 min). The supernatant was filtered with a 0.44 µm filter membrane, and the sample was placed in a balanced DEAE-52 cellulose column (2.6 × 40 cm). After that, deionized water and different gradient concentrations of NaCl solutions (0.1, 0.2, and 0.3 mol/L) were used to elute the sample at a flow rate of 8.0 mL/10 min. At the same time, an automatic collector was used to collect fractions in each tube (8 mL/tube) every 10 min. The phenol-sulfuric acid method was adopted to monitor the polysaccharide content. The eluent with a large absorption peak area was collected, dialyzed with distilled water (3500 Da, 48 h), and freeze-dried for further analysis.

2.7. Determination of monosaccharide compositions

Monosaccharide compositions of US3 and RS3 were determined by HPLC with standard monosaccharides. Briefly, each sample was weighted 20 mg and hydrolyzed with 2 M sulfuric acid (2 mL) at 110 °C for 4 h. After cooling, the hydrolysis was neutralized with barium carbonate and centrifuged to remove the precipitation. Sodium hydroxide solution and PMP were added to the supernatant and standard monosaccharides at 70 °C for 30 min, respectively. After cooling, the solution was neutralized again with HCl, subsequently extracted with chloroform.
until colorless, and then centrifuged. The supernatant was filtered through a 0.22 μm membrane and analyzed by HPLC with the XDB-C18 column. The detection conditions were as follows: the mobile phase was phosphate buffer and acetonitrile (80:20, v/v); the detection wavelength was 245 nm; the flow rate was 1 mL/min; and the injection volume was 10 μL.

2.8. Evaluation of immunomodulation activity

RAW 264.7 macrophages were cultured in DMEM containing Fetal bovine serum (10%), penicillin (100 IU/mL), and streptomycin (100 mg/L) at 37 °C in a 5% CO₂ atmosphere.

MTT method was used to evaluate the proliferation of RAW 264.7 cells. 100 μL cell supernatant (3 × 10⁵ cells/mL) was seeded in 96-well plates and incubated for 12 h. After the adherence of the cells, the medium was discarded, and the cells were treated with 100 μL of different samples (US1, US2, US3, RS1, RS2, and RS3) at different concentrations (25, 50, 100, 200, 400, 800 μg/mL) for 24 h. Each dosage was set in six parallel wells. After cultivation, the supernatants were collected to determine the contents of IL-6 and TNF-α. The lipopolysaccharide (1 μg/mL) was used as a positive control. 100 μL MTT (1 mg/mL) was added to each well and additional incubation for 4 h. Then, the yellow supernatant was sucked up. 100 μL dimethyl sulfoxide (DMSO) was added to each well and incubated for 10 min. The absorbance was determined at 570 nm to evaluate the cell proliferation ratio. The DMEM medium was set as the control group.

2.9. Statistical analysis

All experiments were repeated at least three times. Results were expressed as means ± SD. Statistically significant (p < 0.05) were compared using a one-way analysis of variance (ANOVA) performed by SPSS version 16.0 (IBM Corp., NY, USA). Origin Software Version 2018 (Origin Lab Corp., MA, USA) was used to draw all graphs.

3. Results and discussion

3.1. Effects of ultrasonic mode and frequency on polysaccharide extraction

In this study, four ultrasonic working modes (ESU, ESDU, EADU, DTU) and different combinations of ultrasonic frequencies were studied to evaluate the extraction yield of LMP. The results showed in Fig. 1. The content of LMP by ultrasonic-assisted extraction was significantly higher than that of hot water extraction. Meanwhile, the extraction time in ultrasound was sharply less than that in hot water. As shown in Fig. 1(a), when the energy aggregation counter flow single-frequency ultrasound was performed at 28 kHz, the yield of LMP rapidly increased. However, it gradually decreased with the increase of frequency. According to Fig. 1(b and c), EADU was better than ESDU for enhancing the yield of LMP. There was no significance in the content of LMP among different frequency combinations in either ESDU or EADU. Although the extraction rate was reduced by 1.17%, EADU with frequency combinations of 20/35 kHz was a 10-minute shorter extraction time than 20/28 kHz. Its extraction rate was 18.23% higher than traditional hot water extraction.

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liquid, which is more suitable for extracting multi-target materials [17]. Although there is no wave superposition, ultrasound with alternative frequencies has unique advantages. For instance, the force generated by different frequencies varies, and the probability of the destruction of objects treated by the alternating action of different forces will significantly increase. The blasting of cavitation bubbles generated by the previous frequency ultrasound could not only provide new cavitation nuclei for itself. Additionally, it could offer cavitation nuclei to the subsequent frequency ultrasound, thus increasing the ultrasonic cavitation rate [18].

As can be seen from Fig. 1, the yield of LMP fluctuated to different degrees during the process of ultrasound-assisted extraction compared with hot water extraction, which may be because temporary polymerization was promoted in this process. In short, in the extraction process, energy aggregation ultrasound was better than divergent ultrasound, and alternating dual-frequency ultrasound was better than single frequency and synchronous dual-frequency ultrasound. Therefore, 20–35 kHz EADU was selected as the best ultrasound working mode, and the best extraction time was 15 min.

Fig. 2. Effect of different extraction parameters on the yield of LMP. (a) material to water ratio, (b) ultrasonic power intensity, (c) intermittent time ratio, (d) extraction temperature and spectrum of FT-IR of US1 and RS1(e).
3.2. Effect of single factors on LMP

The effect of different ratios of material to liquid on the content of LMP was investigated when the ultrasound power intensity was 250 W/L. The temperature was 50 °C, ultrasonic intermittent time ratio was 4 s/3 s. The results shown in Fig. 2a, the yield of LMP increased to the highest when the ratio of material to liquid was 1:30 g/mL. The LMP content increased gradually with the increased ratio of material to liquid from 1:10 to 1:30 g/mL, and then the yield of LMP had an upward tendency. It might be attributed that the increasing ratio of liquid to the material could enhance the concentration difference between the interior cells and the exterior solvent, and the cells collapsed under the action of ultrasonic vibration, which promoted the dissolution rate of LMP. On the other hand, more solvent indicated that the cavitation effect was relatively weak to the unit volume, leading to a decrease in LMP content [19]. There was no significant difference in the yield of LMP when the ratio of material to liquid was in the range of 1:10 to 1:30 g/mL. It was also considered that there was severe pollution of high energy consumption in the process of water concentration after extraction, so the optimal ratio of material to liquid of 1:10 g/mL was selected in this study.

Ultrasonic power intensity was one of the significant factors that influenced the yield of LMP. The diverse ultrasound power densities of 100, 150, 200, 250, and 300 W/L were studied when the other extraction conditions were mentioned above. Fig. 2b illustrated the effect of ultrasonic power intensity on the LMP. It was obviously seen that the yield of LMP kept a moderately upward tendency with the increase of ultrasound power intensity, and it reached a top peak at 250 W/L. The rise of LMP content was due to the power-producing a large of cavitation bubbles that created high shear and microjets to disrupt cells and enhance mass transfer [20]. Afterward, the yield of LPM dropped sharply because of the hydrolyzation of polysaccharides [21]. Based on the analysis, although the yield of LMP reached the maximum at 250 W/L, the LMP content only increased by 1.56% compared with 150 W/L.

Fig. 3. The elution curves of US3 (a) and RS3 (b) were eluted with a DEAE-52 cellulose column and the UV spectrum analysis (c).
Hence, the ultrasonic power intensity of 150 W/L was chosen as the optimal condition.

The effect of ultrasonic intermittent time ratio (2 s/3 s, 4 s/3 s, 6 s/3 s, 4 s/1 s, and 4 s/6 s) on the yield of LMP were evaluated when other extraction conditions were unchanged. As shown in Fig. 2c, the extraction yield of LMP significantly increased as the intermittent time ratio was 4 s/3 s, while there was no significance among other intermittent time ratios. The two kinds of cavitation bubbles generated by 20/35 kHz of EADU provided various ways of force on the material. At the same time, the intermittent time could increase the cavitation...
The effect of extraction temperature (20–60 °C) on LMP was investigated, with other conditions remaining unchanged. It can be found in Fig. 1d that the yield of LMP increased slowly from 20 °C to 40 °C, and then it rose rapidly with the temperature at 50 °C. Afterward, the extraction yield leveled off. The yield of polysaccharides increased with the increase in temperature. It may be because higher temperatures loosed the cell walls of the material, and it is easier to break by ultrasonic cavitation, promoting the dissolution of polysaccharides and accelerating the diffusion rate of cell contents [18]. Given economic and nutrition, 50 °C was selected as the optimization extraction temperature.

3.3. Effect of ultrasound on the extraction rate of LMP

When the ratio of material to liquid was 1:10 g/ml, the ultrasonic power intensity was 150 W/L, the intermittent time ratio was 4 s/3 s, the extraction temperature was 50 °C, and the extraction time was 15 min, the extraction rates of different components (US1, US2, US3, RS1, RS2, and RS3) after freeze-drying was determined. The results showed that the extraction of polysaccharides by ultrasonic assisted extraction US1, US2, US3 were 54.85%, 18.12%, 8.2%, respectively, and the extraction of polysaccharides by hot water extraction RS1, RS2, RS3 were 49.52%, 14.00%, 5.66%, respectively. Compared with the traditional hot water extraction, the extraction rates of US1, US2, and US3 increased by 10.80%, 29.40%, and 44.90%, respectively, which may be due to a complete disintegration of the cell wall by ultrasound [12]. Therefore, this ultrasonic-assisted extraction had the advantages of short extraction time, low energy consumption, and high yield, which provided a basis for the industrial promotion of maca polysaccharide extraction.

3.4. FT-IR analysis

Infrared spectroscopy is a powerful qualitative analysis technique of organic functional groups, which is used to clarify the structure of polysaccharides. FT-IR of US1 and RS1 was performed, and the results showed...
were shown in Fig. 2. The FT-IR spectra at approximately 3226 cm\(^{-1}\) were a broadly-stretched intense peak representing O–H stretching vibrations in the hydrogen bonds of intermolecular and intramolecular. An absorption peak at 2926.87 cm\(^{-1}\) was ascribed to the presence of asymmetric bending vibration caused by the C–H bond [22]. Besides, the absorption at 1613 cm\(^{-1}\) belonged to the asymmetric stretching vibration of the C=O bond, while the peak at about 1405 cm\(^{-1}\) reflected C–H bending [10]. The strong band between 1200 and 1000 cm\(^{-1}\) was caused by the overlapping of ring vibration, the stretching vibrations of C-O-H side groups, and C-O-C glycosidic band vibrations, indicating the possible presence of pyranose. The absorption band at 923 cm\(^{-1}\) represented the stretching vibration of β-glucopyranoside, while the peak at around 845 cm\(^{-1}\) was on behalf of α-type glycosidic linkage [19]. The results of FT-IR analysis suggested that the absorption peaks of US1 and RS1 were consistent, indicating that the structure of LMP was unchanged after the ultrasonic-assisted extraction.

3.5. Isolation and purification of US3 and RS3

US3 and RS3 were further purified by DEAE-52 cellulose column anion exchange to obtain the target component. The process mainly involves removing a material's ions by ion exchange with another...
material. The commonly used eluents are ultrapure water, alkali solutions, and salt solutions of different concentrations. The samples are separated from the target components by the eluent. In separating polysaccharide samples, neutral sugars with high water solubility are eluted out first, whereas acidic sugars with poor water solubility are eluted out last. The elution curves were displayed in Fig. 3. After gradient elution with deionized water, 0.1, 0.2, and 0.3 M NaCl solution successively, two major peaks were obtained, including one peak of water-eluted neutral polysaccharide and a peak of 0.1 M NaCl eluted acidic polysaccharide. The main component eluted with deionized water was collected for dialysis overnight and freeze-dried to obtain white flocculent powder polysaccharide. The neutral polysaccharides purified by hot water and ultrasound were named MK-R and MK-C, respectively. The extraction rate and purity of MK-R and MK-C were 10%, 95.6%, and 10%, 93.34%, respectively.

MK-C and MK-R were prepared in 1.5 mg/mL solution, respectively, which were analyzed by a UV spectrophotometer over the range from 200 nm to 800 nm, the results shown in Fig. 4. There was only an absorption peak at around 200 nm, which indicated the presence of LMP. Furthermore, there was no absorption peak in the range of 260 nm to 280 nm, demonstrating that MK-C and MK-R did not contain nucleic acid, protein, polypeptides, and other impurities.

3.6. Monosaccharide composition

HPGPC was used to analyze the monosaccharide compositions. The results of standard monosaccharides, MK-C, and MK-R are shown in Fig. 5. Both MK-C and MK-R consisted of arabinose, galactose, and glucose with the molar ratios of 2.9:2.72:5.05 and 3.5:3.7:7.35, respectively. Glucose was the major monosaccharide, which was consistent with the results of Li et al. [4]. Zhang et al. [14] showed that a novel polysaccharide isolated from maca consisted of arabinose, mannose, glucose, and galactose, while Wang et al. [13] determined the polysaccharide (MP21) contained rhamnose, arabinose, and galactose. The main reason for the differences between the different monosaccharide compositions may be attributed to the raw material and the detection methods.

3.7. Cell viability test

The toxicity and safety of preselected compounds evaluated by setting various concentrations play a crucial role in biological activity [23]. RAW 264.7 cells were treated with different samples (US1, RS1, US2, RS2, US3, RS3) and different concentrations (25, 50, 100, 200, 400, and 800 ug/mL) for 24 h, and the cell viability was determined by MTT method. As shown in Fig. 6, there was no lethal effect on macrophages with the treatments of samples with different concentrations compared with the control. Furthermore, these samples at various concentrations from 25 to 800 ug/mL significantly promoted the growth of RAW 264.7 cells. However, it did not appear to be an increasing relationship with the increase of sample concentration but an irregular dose-dependent relationship.

3.8. Immunomodulation activity

Cytokines, a series of intercellular signaling proteins, are stimulated by both the immune and non-immune systems, such as TNF-α and IL-6, which form the complex regulatory network systems. They control the homeostasis by induction of cell differentiation, proliferation, apoptosis, and inflammatory response. The induction of cytokine synthesis is a major approach to assessing the augmentation activity of innate immunity [13]. ELISA assays were used to determine TNF-α and IL-6 levels in the supernatant of RAW 264.7 treated with different samples (US1, RS1, US2, RS2, US3, RS3) with different concentrations (25, 50, 100, 200, 400, and 800 ug/mL). The results are displayed in Fig. 7. It can be seen that US3 and RS3 had the most prominent release effect on TNF-α and IL-6, which could achieve the best immunomodulation effect at the low concentration. It is well known that the immunomodulatory activity of polysaccharides has been widely reported to be associated with the chemical composition, molecular weight, conformation, glycosidic bond, and degree of branching of polysaccharides [24]. The purified polysaccharides exerted the best immune activity compared with crude polysaccharides and crude extract because the active sites of the polysaccharide were fully exposed, which could strongly activate macrophages to secrete a large number of cytokines. In addition, the effect of US3 on cytokine production was more effectively activated macrophages than that of RS3. It may be due to the fact that ultrasonic waves caused the vibration of the solid-liquid interface, which improved the extraction rate of polysaccharides and changed the structure of polysaccharides [25].

4. Conclusions

In this study, three types (ESDU, EADU, and DTU) of ultrasonic working modes were applied to evaluate the extraction efficiency of maca polysaccharides. The results showed that the optimized ultrasonic working mode was EADU with frequency combinations of 20/35. Compared with traditional hot water extraction, the extraction rates of crude extract, crude polysaccharides, and purified polysaccharides increased by 10.80%, 29.40%, and 44.90%, respectively. Furthermore, the polysaccharide structure was not destroyed after the ultrasonic-assisted extraction. The monosaccharide compositions were composed of arabinose, galactose, and glucose, which are considered to have high biological activity. In addition, US3 showed a significant immune activity by promoting the secretion of cytokines (TNF-α and IL-6) in RAW 264.7. This study provided a new idea for the industrial extraction of maca polysaccharides by multimodal ultrasound. And the purified maca polysaccharides could be explored as a kind of immunizing agent in the food or pharmaceutical industries. The mechanism of immunomodulatory activity and the structure-function relationships of maca polysaccharides will be investigated in further study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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