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Maca polysaccharides: Extraction optimization, structural features and anti-fatigue activities

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ABSTRACT

The maca polysaccharides optimal extraction conditions were obtained by using response surface methodology (RSM) method and the anti-fatigue activity of maca polysaccharides (MCP) was explored. The maca polysaccharides extract yield of RSM could reach 9.97 mg/g by using the model predicts, and the total sugar and protein purity were 61.00% and 4.46% with the further isolation process, respectively. And the monosaccharide compositions obtained by gas chromatograph (GC) were composed of rhamnose (rha), glucose (glc), galactose (gal) with the ratio of 2.34:10.21:1.00. Furthermore, the anti-fatigue activity was evaluated by the swimming parameter, biochemistry parameters (liver glycogen (LG), blood urea nitrogen (BUN), and lactic acid (LD)), the result indicated that the low-dose maca polysaccharides group had the significant anti-fatigue activity.

1. Introduction

Maca, *Lepidium meyenii* (Walp.), is used as functional food and medicine due to the multiple bioactive effects, including, anti-fatigue activity, sexual and fertility enhancement, and memory impairment [1–5]. The main biological components isolated from maca are polysaccharides, alkaloids, and polyphenols [6,7].

As one of the main component and important bioactive ingredient, maca polysaccharides have attracted great attention. The researches of maca polysaccharides usually consisted of extraction and purification methods, structural analysis and pharmacological effects. The general methods of extraction are water extraction [8] and ultrasonic circulating extraction [9], and the general methods of isolation and purification are alcoholic precipitation and resin purification (ion-exchange column and gel filtration chromatography). The maca polysaccharides obtained were further analyzed to get the structural characteristics, and the pharmacological activities of maca polysaccharides were studied, included anti-fatigue activity [8], anti-oxidant activity [10], immunomodulatory effect [11] and liver protective effect [9]. Zha et al. studied the crude polysaccharides with different ethanol concentration precipitation, the result indicated that maca polysaccharides had anti-oxidant activity and the monosaccharide compositions were composed of rhamnose, arabinose, glucose and galactose [10]. Wang et al. obtained the maca polysaccharides (MP21) by water extraction and ethanol precipitation and was further isolated by the column separation (DEAE-52 cellulose column and Sephacryl™ S-500 column), and was further confirmed that MP21 could enhance macrophage activities [11] (Table 1).

Fatigue is the commonly existed performance, the reasons that caused the phenomenon can be summarized as followed (1) Energy sources consumption and depletion. (2) Metabolic products production and accumulation. (3) Immune system dysfunction. (4) Reactive oxygen species (ROS) excessive generation and the cellular structure damage [12–15]. Many researches attempted to find natural anti-fatigue ingredients to delay fatigue, improve athletic ability without adverse effects [16]. The anti-fatigue activity of maca polysaccharides has great reported, such as, two fractions of maca polysaccharides (MPS-1 and MPS-2) [8], maca polysaccharides (MP) [17], which mainly focus on the high-purity compounds. The purification processes were complicated, which was not well used in the industrial production (Table 1).

The abundant previous researches enriched the maca polysaccharides activities. However, the incomplete extraction methods and the difficult purification methods limited the maca polysaccharides' development in industry. In all, the paper studied the best water extraction technology by response surface methodology (RSM) application and ethanol precipitation method, in order to make maca polysaccharides products have more wide development in industry and market, the paper studied the maca crude polysaccharides' (MCP) anti-fatigue activity for the further develop in industrial production.

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Table 1
The summary anti-fatigue effects of maca polysaccharide compositions.

Polysaccharide composition	Isolation and purification method	Purify	Nature	Monosaccharide composition	References
Maca polysaccharide (MCP)	Water extraction and ethanol precipitation	61.00%	Heteropolysaccharide	D Ara, D xyl, D Man, D glc, D gal, with the molar ratio of 7:2:4:36:6	In this paper
Maca polysaccharides at final ethanol concentration of 60%–90% (LMP-60-90)	Water extraction and ethanol precipitation	39.5%–69.4%	Heteropolysaccharide	Rha, Ara, Glc, Gal	[10]
Maca polysaccharide (MPS-1)	Water extraction; DEAE-52 cellulose and Sephadex C-100 column	93.2%	Neutral polysaccharide	Xyl, ara, gal and glc, with the mole ratio of 1:1.7:3.3:30.5	[8]
Maca polysaccharide (MPS-2)	Water extraction; DEAE-52 cellulose and Sephadex C-100 column	91.5%	Acidic polysaccharide	Ara, gal and glc, with the mole ratio of 1:1.3:36.8	[8]
Maca polysaccharides (MP)	Sephacryl S-100 HR	99.20%	Acidic polysaccharide	D GalA, D glc, L ara, D man, D gal and L rha, with the molar ratio of 35.07:29.98:16.98:13.01:4.21:0.75	[17]
MC-1	Water extraction; DEAE-Sepharose; Sephadex G-100	97.5%	Neutral polysaccharide	Ara, man, glc, gal, with the molar ratio of 26.21:11.81:53.66:8.32	[18]
MC-2	Water extraction; DEAE-Sepharose; Sephadex G-50	–	Neutral polysaccharide	Ara, man, glc, gal, with the molar ratio of 20.9:4.5:71.9:2.7	[19]
MP-21	Water extraction; DEAE-52; SephacrylTM S-500	90.5%	Neutral polysaccharide	Rha, ara, gal, with the molar ratio of 1:4.84:5.34	[11]
MP-1	Ultrasonic circulating extraction; DEAE-52	91.63%	Acidic polysaccharide	Rha, galA, glc, gal, xyl, ara	[9]

2. Materials and methods

2.1. Materials and reagents

Maca tubers were purchased from Yantai supplier, Shandong, China. And the samples of maca were obtained from Yunnan province of China during in November–December at 2800–3200 m sea level. And the samples were identified by Prof. Demin Gao (Shandong University of Traditional Chinese Medicine, Jinan, China). The tubers were washed, sliced, and powdered by a pulverizer and passed through a 40-mesh sieve and stored in the room temperature condition. The biological activities and structures of maca are highly correlated with the origin.

Rhamnose (rha), fucose (fuc), arabinose (ara), xylose (xyl), mannose (man), glucose (glc), galacturonic (gal), glucuronic acid (glcA), and galacturonic acid (galA) were obtained from Sigma Chemical Co., Ltd. Phenol, blood urea nitrogen (BUN), lactic acid (LD) and liver glycogen (LG) kits, were acquired from Nanjing Jiancheng Bioengineering Institute. Ethanol, chloroform, acetone, *n* butyl alcohol were purchased from Sinopharm Chemical Reagent Co., Ltd. Trifluoroacetic acid was purchased from Aladdin Industrial Corporation.

2.2. Preparation and determination of maca polysaccharide extract (MPE)

Based on the single factor experiment results, each dried maca powder (5.0 g) and water solution were extracted 2 times at different liquid to solid ratio (20:1–30:1 mL/mg). Then the heating procedure was conducted in the different extraction temperature (70 °C–90 °C) for various extraction time (2.5 h–3.5 h). The extract was filtered and centrifuged at 4000 rpm for 10 min and then the supernatant was collected. The MPE content of part collection was measured by the phenol-sulfuric acid method [11]. The yield of MPE (Y) was measured as followed,

$$Y = \frac{C \times V}{m} \times 100\% \quad (1)$$

where C is the extract MPE concentration (mg/mL), V is the extract MPE volume (mL), m is the dried maca powder mass (mg).

Based on the best extraction conditions, the extract was condensed at the solution to raw material (2:1), and ethanol precipitation to a concentration of 80% and placed at 4 °C for 24 h, the precipitation was collected and washed at ethanol 3 times and acetone 3 times, respectively. And the supernatant was deproteinized by improved Sevag's method (solution to Sevag's reagent = 1:1, 1 times; solution to Sevag's reagent = 4:1, 7 times). The supernatant was precipitated at final ethanol concentration of 80% and stored at 4 °C overnight. The precipitate was collected and washed with acetone and petroleum ether in turn, and then was dried at 60 °C to obtain MCP.

2.3. RSM experimental design

In order to obtain the best extraction conditions of MPE, the RSM was used to evaluate optimized technology based on the pre-experiment results. The response value was extraction yield, and the variables (extraction temperature, extraction time and liquid to solid ratios) levels were coded +1, 0 and −1 respectively according to the following formula.

$$X_i = \frac{X_i - X_0}{\Delta X_i}, i = 1, 2, 3 \quad (2)$$

where X_i is the coded values; x_i is the independent parameter practical value; x_0 is the independent parameter real value at the center point; Δx_i is the step change value.

The detailed experimental matrix was listed in Tables 2 and 3. And the system model was employed as mentioned above.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (3)$$

where Y is the predicted response value, β_0 is the intercept term, β_i, β_{ii}, and β_{ij} are the linear term, squared term and interaction term, respectively, X_i and X_j both are the independent parameter coded levels ($i \neq j$).

Table 2
Levels and variable code for RSM.

Independent variables	Symbols	Coded levels		
		−1	0	+1
Extraction temperature (°C)	X_1	70	80	90
Extraction time (h)	X_2	2.5	3	3.5
Liquid to solid ratios (mL/mg)	X_3	20:1	25:1	30:1

Table 3
The responses for MPE yields.

Run	Coded values			Y	
	X_1	X_2	X_3	Experimental	Predicted
1	90	2.5	25	4.79	4.74
2	80	3.5	30	4.29	4.65
3	70	2.5	25	7.82	8.34
4	80	3	25	9.71	9.49
5	80	3	25	9.65	9.49
6	70	3.5	25	5.06	5.11
7	80	3	25	9.65	9.49
8	90	3.5	25	4.57	4.05
9	80	3	25	9.27	9.49
10	70	3	20	7.25	7.09
11	80	2.5	20	8.79	8.43
12	80	3	25	9.16	9.49
13	70	3	30	6.13	5.72
14	90	3	20	4.79	5.20
15	80	3.5	20	7.52	7.63
16	80	2.5	30	7.9	7.79
17	90	3	30	2.78	2.94

The results of RSM were evaluated by the Design-Expert 8.0.5b software. The significance of statistics analysis was evaluated by analysis of variance (ANOVA). *F*-value and *p*-value are used to access the regression coefficient significance and coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{Adj}) are used to express the adequacies of models. The surface and 3D contour plots are applied to demonstrate the relationship between the independent parameters and responses [20,21].

2.4. Physicochemical characterizations of MCP

2.4.1. Determination of MCP and protein contents

The total sugar and protein contents of optimal extraction and purify process were measured by the phenol-sulfuric acid and Bradford method, respectively [22,23].

2.4.2. UV analysis

The UV spectrophotometer (UV9100B) was used to measure the spectrum over the range from 200 nm to 800 nm and explore the protein and amino acids existence [24].

2.4.3. Analysis for monosaccharide compositions of MCP

The monosaccharide compositions of MCP were determined by the gas chromatograph (GC), the detailed process was conducted as follows: 30 mg MCP was hydrolyzed with 5 mL 2 mol/L trifluoroacetic acid solution (TFA) at 120 °C for 1.5 h, and then the methanol was added to remove the excess TFA and repeated 3–4 times. MCP was further analyzed and detected by the GC instrument equipped with DB-17 high-performance capillary column (30 m × 0.25 mm, 0.25 μm). The vaporizer temperature was 250 °C and detector temperature was 280 °C. And the column temperature procedure was showed as followed, 180–210 °C, 5 °C/min → 210–215 °C, 0.3 °C/min → 215–240 °C, 8 °C/min. The external calibration was used to evaluate the MCP monosaccharide compositions.

2.5. Anti-fatigue activity

2.5.1. Experiment design

40 male Kunming mice that were purchased from Pengyue Experimental Animal Center (Shandong, China) and were housed under the conditions, temperature was 25 ± 1 °C, humidity was 50%–60%, and the standard diet and water were free obtained. The animals were randomly divided into four groups after 7 d acclimatization: normal group, MCP low-dose group (MCP-L) (150 mg/kg·d), MCP middle-dose group (MCP-M) (300 mg/kg·d), MCP high-dose group (MCP-H) (600 mg/kg·d). The animals were administrated oral treatment with dose of 0.15 mL/10 g at 9:00–10:00 am every morning for 30 days.

2.5.2. Weight analysis

The weights of mouse were recorded in the 1st, 8th, 15th, 22th, 30th day. And the data was analyzed by SPSS software.

2.5.3. Forced swimming test

After 30 d consecutive gavage treatment, all of mice swam with a load that was corresponded to 5% of the weights to the tail. The swimming container (length of 35 cm, temperature of 25 ± 1 °C), and the swimming time was recorded until the mice failed to raise the water surface within 5 s.

2.5.4. Analysis of biochemical parameters and organ indices

The mice were immediately anesthetized, and then the organs (liver, spleen, thymus) and blood obtained by removing eyeball and were collected after the forced swimming, and the serum was obtained by centrifugation at 4000 rpm for 10 min. The biochemical indexes (LG, BUN, and LD) were analyzed by the biochemical reagent kits.

2.6. Statistical analysis

The statistics were analyzed by the software SPSS Statistics 17.0, and One-way analysis of variance and Duncan's test were used to compare the group differences. The results were indicated by means ± standard deviation, and $P < 0.05$ was considered that the statistics had significant differences.

3. Results and discussion

3.1. Optimization of MPE

3.1.1. Single factors results

The explored single factors studies were consisted of three parameters (extraction temperature, extraction time, liquid to solid ratio). The optimal conditions based on the single factor assays were: extraction temperature was 90 °C, extraction time was 3 h, and the liquid to solid ratio was 25:1 mL/g.

The general single factor extraction tests were conducted as followed, take the example of extraction temperature single factor test, the extraction temperature was different, while extraction time and liquid to solid ratio were set as 3 h and 25:1 mL/g, respectively. The extraction temperature was within the range from 50 °C to 100 °C, when the extraction temperature increased from 50 °C to 80 °C, the yield increased. In addition, the yield could obtain the highest number (9.435 ± 0.25 mg/g) when the extraction temperature was 80 °C. The increasing of extraction temperature could enhance the diffusion of MPE in extraction solvent, Whereas, the extraction yield dropped when the temperature over 90 °C due to the MPE structure damage and degradation.

The similar trend was performed in the extraction time and liquid to solid ratio single assays. Higher extraction time and liquid to solid ratio could increase the MPE yield due to the MPE dissolution increased, however, when the extraction time exceeded 3 h and liquid to solid ratio exceeded 25:1 mL/g, the yield dropped due to the impurities increased and cavitation energy assimilation [25].

3.1.2. Optimization of MPE yields

The RSM design was applied to evaluate the extraction temperature, extraction time and liquid to solid ratio effects as well as the interaction effects for yields of MPE. The independent variables have been confirmed according to the single-factor assays. The range of independent variables was displayed in Table 2, and the yield of MPE was analyzed

and the results were showed in Table 3. The predicted response Y for MPE yield could be demonstrated by the equation as follows:

$$Y = 9.49 - 1.17X_1 - 0.98X_2 - 0.91X_3 + 0.63X_1X_2 - 0.22X_1X_3 - 0.59X_2X_3 - 2.91X_1^2 - 1.02X_2^2 - 1.34X_3^2 \quad (4)$$

where Y is the MPE predicted yield, X_1 is the extraction temperature coded parameter, X_2 is the extraction time coded parameter, X_3 is the liquid to solid ratio coded parameter.

The different influencing factors of MPE yield were evaluated by Analysis of variance (ANOVA), and the predictive model was displayed in Table 4. The model had a significant influence on MPE yield, which was evaluated by the parameter of *F*-value (model = 41.91) with a low *p*-value ($P > F$) < 0.0001. Lack of Fit "$P > F$" values was >0.05 and model term *p*-value was <0.05, which indicated that RSM results had statistically significant and test fit was good. The *p*-value ($P < 0.05$) of linear coefficients of X_1, X_2 and X_3, cross product coefficients of X_1X_3, X_2X_3, and quadratic coefficients of X_1^2, X_2^2, X_3^2, suggested that the coefficients were significant. In addition, the determination coefficient (R^2) value was 0.9818 and adjusted R^2 was 0.9584, which were closer to 1, demonstrated that the high efficacy of the second-order polynomial equation. In addition, the adequate precision was 6.38, indicated that the model was an adequate signal and could navigate the design space. Besides, the regression model was a good experimental result prediction and the real factor effects.

3.1.3. Analysis of response surface

The three-dimensional response surface plots (3D-surface) were used to show the interactions between experimental levels of each factors. The different plots shapes indicated that the different influences between the different variables. For example, the elliptical counter plots showed the significant influences on the relevant parameters [26]. And the 3D-surface showed that the mutual effects of two parameters, while the other parameter was set at zero level.

The MPE yields (Y) under different extraction temperature (X_1) and extraction time (X_2), while the liquid to solid ratio (X_3) was set at zero level. The MPE yield increased when the extraction temperature in the range from 70 °C to 77.29 °C and the extraction time in the range from 2.5 h to 2.72 h, and the yield decreased when the extraction temperature exceed 77.29 °C and the extraction time exceed 2.72 h. Analysis the reason that caused the phenomenon, which may due to the higher extraction temperature could influence the biochemical activity and the increasing of the polysaccharides decompositions.

The interaction effects between the extraction temperature and liquid to solid ratio, the results did not indicate the significant influence. The highest yield was 9.74 mg/g when the extraction temperature was 78.15 °C and the liquid to solid ratio was 23.37:1 mL/g. The further increased of extraction temperature and liquid to solid ratio had the opposite influence on the MPE yield.

The significant influence between the extraction time and liquid to solid ratios was studied. The yield increased when the extraction time increased from 2.5 h to 2.8 h, and the liquid to solid ratio between 20 mL/g and 23.79 mL/g. The extraction yield not increased until the extraction time was 2.8 h and the liquid to solid ratio was 23.79 mL/g, which may due to the saturation in the solubility of MPE and the enhancement of the impurities.

According to the RSM optimal extraction result, the optimal condition was as follows: extraction temperature was 77.54 °C, extraction time was 2.75 h, and liquid to solid ratios was 23.96:1 mL/g. The predicted extraction yield was 9.97 mg/g at the optimal conditions.

3.1.4. Verification

In order to verify the optimal parameters and levels of the extraction technology, the best optimal extraction conditions were used to be implemented and three parallel trials were conducted. The MPE extraction yield was 9.975 ± 0.021 mg/g and closed to the predicted value, which indicated that the RSM model was suitable for the MPE optimization.

3.2. MCP characterizations

3.2.1. Contents of total sugar and protein

MCP was obtained by the water extraction and ethanol precipitation and the protein was measured by Bradford method to assess the deproteinization result. Based on the optimal extraction results, the purified MCP total sugar and protein contents of optimal extraction were 61.00% and 4.46% based on the dry crude MCP, respectively.

3.2.2. UV analysis

The detailed message was showed in Fig. 1. There was an absorption peak at 207 nm, which demonstrated the existence of maca polysaccharides. What's more, the existence of 280 nm absorption peak revealed that MCP consisted of amino acids. And the absence of the protein absorption peak was not consistent with the Bradford assay result, which may due to the protein had denaturation and inactivation.

3.2.3. MCP monosaccharide composition

GC was used to analyze the monosaccharide compositions, the result demonstrated that MCP consisted of Rha, Glc and Gal, with the molar ratios of 2.34:10.21:1.00. And glucose is the major monosaccharide composition of MCP heteropolysaccharide. At the similar experiment of Zha et al., the monosaccharide compositions of maca polysaccharides were consisted of D rha, D ara, D glc and D gal, and D gal was the highest content of maca monosaccharide compositions [27]. The differences between the different monosaccharide compositions maybe due to the

Table 4
ANOVA for RSM for maca polysaccharides.

Source	Squares	df	Square	F-value	Prob > F	Significant
Model	80.09	9.00	8.90	41.91	<0.0001	***
A-extraction temperature	10.88	1.00	10.88	51.25	0.0002	***
B-extraction time	7.72	1.00	7.72	36.37	0.0005	***
C-liquid to solid ratios	6.57	1.00	6.57	30.95	0.0008	***
X_1X_2	1.61	1.00	1.61	7.60	0.0282	*
X_1X_3	0.20	1.00	0.20	0.93	0.3663	
X_2X_3	1.37	1.00	1.37	6.45	0.0387	*
X_1^2	35.60	1.00	35.60	167.68	<0.0001	***
X_2^2	4.38	1.00	4.38	20.64	0.0027	**
X_3^2	7.59	1.00	7.59	35.76	0.0006	***
Residual	1.49	7.00	0.21			
Lack of fit	1.23	3.00	0.41	6.38	0.0527	
Pure error	0.26	4.00	0.06			
Cor total	81.57	16.00				

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

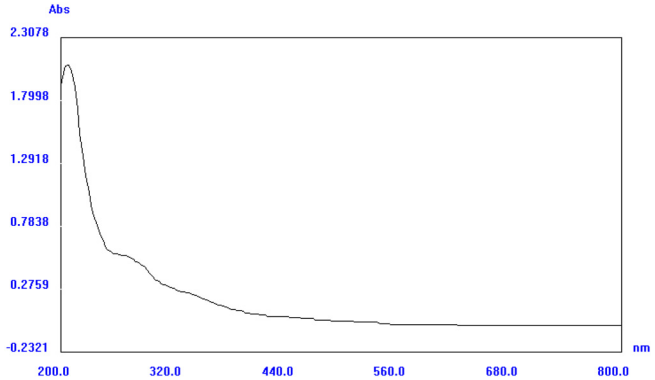

Fig. 1. The UV spectrum analysis.

differences between the raw material and the detection methods. The detailed information was shown in Fig. 2.

3.3. MCP anti-fatigue activity

3.3.1. Weight and organ index analysis results

The mouse weight variation trend was recorded in Fig. 3. Compared to the control group, the MCP-L, MCP-M, MCP-H group did not show the significant differences ($P > 0.05$). And there were no significant differences in the spleen index and the thymic index between the control group and experimental groups ($P > 0.05$), which revealed that the anti-fatigue activity was not related with the immunocompetence. And the liver index result ($P > 0.05$) indicated that the mouse with MCP did not show the side reaction during the feeding period.

3.3.2. Exhaustive swimming time result

Exhaustive swimming time is the direct motion index to evaluate the anti-fatigue ability. By comparing the exhaustive swimming time between the control group and different dose MCP treatment groups, the result indicated that MCP had a significant influence ($P < 0.05$) on anti-fatigue activity. The results were showed in Fig. 4. And the MCP-L, MCP-M, MCP-H group increased the ratios of exhausting swimming time were 198%, 148%, 177%, respectively. The order of the MCP group significant level was, MCP-L group > MCP-H group > MCP-M group, which did not show the dose-dependent relationship.

3.3.3. Biochemical parameters results

The fatigue performance was caused due to the energy consumption and deficiency, and it can reduce the body endurance capacity. Energy can be generated by the glycogen breakdown [28] and circulating glucose production. The content of increasing LG indicated the anti-fatigue ability improved. The result showed that MCP-L group had more significant influence than that of the control group, and higher than that of the control group by 15.61%.

As the metabolic product of protein and amino acid metabolism, BUN is the important index to evaluate the fatigue activity [29]. The MCP-L group could significantly decrease the BUN level compared to the control group in mice ($P < 0.05$). However, the differences between the MCP-M, MCP-H groups and the control group were not significant on reducing BUN level.

LD is the glycolysis product under anaerobic condition, the accumulation of it can be harmful to the organs and lead to the fatigue [30,31]. The LD content had not shown the significant difference between MCP groups and control group on the LD level, which may due to the individual differences (Fig. 5).

In summary, the biochemical parameters (LG, BUN and LD) were chose as indexes to evaluate the anti-fatigue activity. And three of the parameters had significantly differences compared to the control group, the result indicated that the MCP had anti-fatigue effect. The result indicated that the MCP-L group significantly increased the LG level and decreased the BUN level and did not show the dose-dependently relationship. And the LG parameter was the most significant index to evaluate the anti-fatigue activity among the four parameters. The anti-fatigue effect may be due to the increasing of LG level, and it related with the MCP composition structures. According to the previous papers, we found that the neutral polysaccharide and acidic polysaccharide isolated from maca had the anti-fatigue activity, implied that the activity of MCP maybe attribute to the synergistic effect [8]. However, based on the healthcare production and industrial production, the simple extraction and purification process was beneficial for the further production. In all, the paper conducted the anti-fatigue experiment with MCP at the optimal extraction conditions. In addition, the papers of anti-fatigue activity had explored the relationship between the anti-fatigue activity and anti-oxidant activity, demonstrated that the mechanism was related with the oxidative stress [32]. However, the serum parameters (MDA, GPx and SOD) did not show the significant influences between the experiment groups and the control group. The mechanism of anti-fatigue activity still need to be further explored.

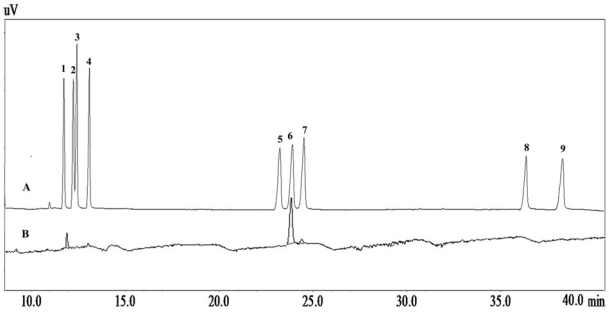

Fig. 2. Standard monosaccharide mixture (A) and MCP sample (B). 1: Rha, 2: Fuc, 3: Ara, 4: Xyl, 5: Man, 6: Glc, 7: Gal, 8: GlcA, and 9: GalA.

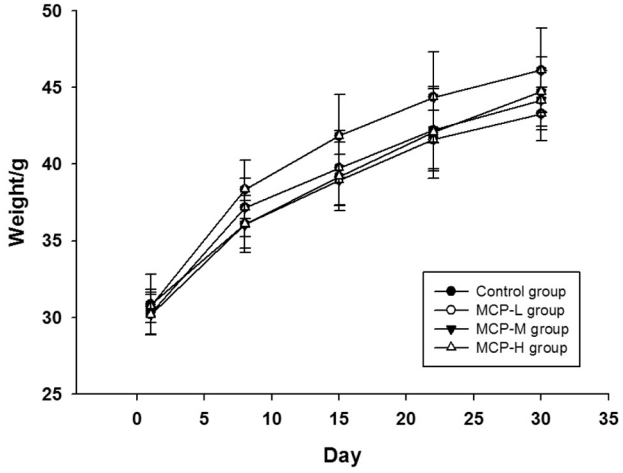

Fig. 3. Effects of MCP on weight variation trend in mice. MCP-L: MCP low dose group; MCP-M: MCP middle dose group; MCP-H: MCP high dose group. Every column represents the means ± standard errors. *, $P < 0.05$; **, $P < 0.01$.

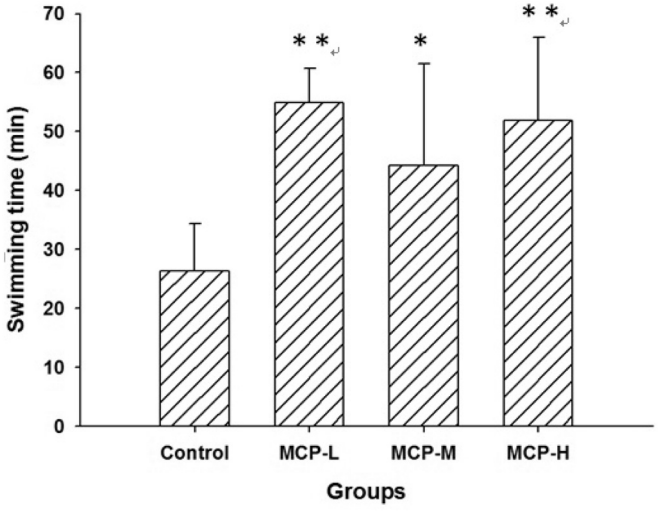

Fig. 4. Effects of MCP on forced swimming time in mice. MCP-L: MCP low dose group; MCP-M: MCP middle dose group; MCP-H: MCP high dose group. Every column represents the means ± standard errors. *, $P < 0.05$; **, $P < 0.01$.

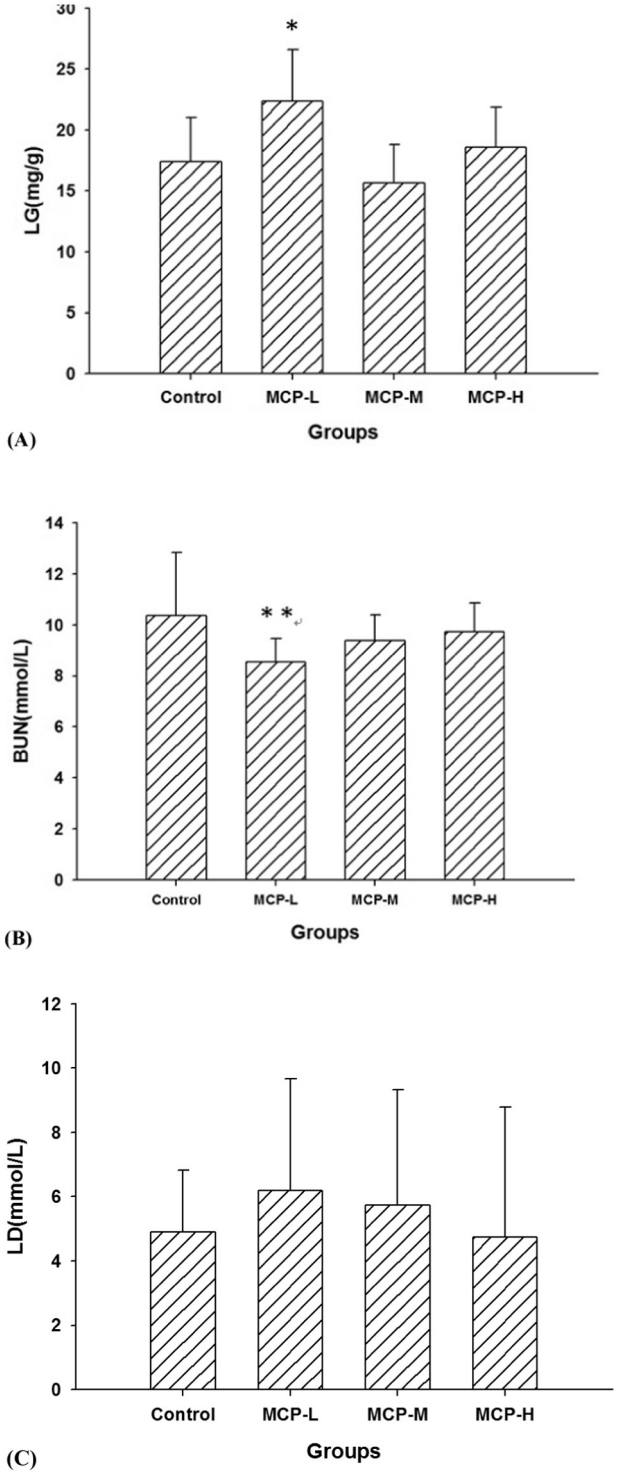

Fig. 5. Effects of MCP on LG (A), BUN (B), and LD (C) in mice. MCP-L: MCP low dose group; MCP-M: MCP middle dose group; MCP-H: MCP high dose group. Every column represents the means ± standard errors. *, $P < 0.05$; **, $P < 0.01$.

4. Conclusions

MPE was obtained by the water boiling extraction and the optimal extraction conditions by RSM were studied. The result was listed as follows, extraction temperature was 77.54 °C, extraction time was 2.75 h, and liquid to solid ratio was 23.96 mL/mg, and the predicted yield could reach 9.97 mg/g. The further ethanol precipitation and Sevag deproteinization method to purify maca polysaccharides, and purify of total sugar and protein were 61.00% and 4.46% respectively. And the monosaccharide compositions were composed of rhamnose (rha), glucose (glc), galactose (gal), at the ratio of 2.34:10.21:1.00. Maca was usually used as healthcare product, however, the main material was mainly extract and powder, the substance-function relationship was unclear. The paper aimed to show the maca polysaccharide's function of anti-fatigue and further provided the raw material of healthcare product. The swimming parameter and biochemical parameters results showed that MCP had anti-fatigue activity. In addition, the optimal extraction condition could increase the crude MCP yield and be beneficial for the industrial production.

Conflict of interest

The authors did not declare conflict of interest.

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References

[1] Y. Wang, Y. Wang, B. McNeil, L.M. Harvey, Maca: an Andean crop with multi-pharmacological functions, Food Res. Int. 40 (2007) 783–792.
[2] E.H. Choi, J.I. Kang, J.Y. Cho, S.H. Lee, T.S. Kim, I.H. Yeo, H.S. Chun, Supplementation of standardized lipid-soluble extract from maca (*Lepidium meyenii*) increases swimming endurance capacity in rats, J. Funct. Foods 4 (2012) 568–573.
[3] G.F. Gonzales, A. Cordova, K. Vega, A. Chung, A. Villena, C. Gonez, Effect of *Lepidium meyenii* (Maca), a root with aphrodisiac and fertility-enhancing properties, on serum reproductivehormone levels in adult healthy men, J. Endocrinol. 176 (2003) 163–168.
[4] D. Massoma Lembe, M. Gasco, G.F. Gonzales, Fertility and estrogenic activity of *Turraeanthus africanus* in combination with *Lepidium meyenii* (black maca) in female mice, Eur. J. Integr. Med. 4 (2012) e345–51.
[5] J. Rubio, W. Qiong, X. Liu, Z. Jiang, H. Dang, S.L. Chen, G.F. Gonzales, Aqueousextract of black maca (*Lepidium meyenii*) on memory impairment induced byovariectomy in mice, Evid. Based Complement. Alternat. Med. 2011 (2011), 253958. .
[6] J. Zhao, I. Muhammad, D.C. Dunbar, J. Mustafa, I.A. Khan, New alkamides from Maca (*Lepidium meyenii*), J. Agric. Food Chem. 53 (2005) 690–693.
[7] N. Bai, K. He, M. Roller, C. Lai, L. Bai, M. Pan, Flavonolignans and other constituents from *Lepidium meyenii* with activities in anti-inflammation and human cancer cell lines, J. Agric. Food Chem. 63 (2015) 2458–2463.
[8] J. Li, Q. Sun, Q. Meng, L. Wang, W. Xiong, L. Zhang, Anti-fatigue activity of polysaccharide fractions from *Lepidium meyenii* Walp (maca), Int. J. Boil. Macromol. 95 (2017) 1305–1311.
[9] L. Zhang, Q. Zhao, L. Wang, M. Zhao, B. Zhao, Protective effect of polysaccharide from maca (*Lepidium meyenii*) on Hep-G2 cells and alcoholic liver oxidative injury in mice, Int. J. Biol. Macromol. 99 (2017) 63–70.
[10] C.C. Kang, L.M. Hao, L.M. Zhang, Z.Q. Zheng, Y.W. Yang, Isolation, purification and antioxidant activity of polysaccharides from the leaves of maca (*Lepidium Meyenii*), Int. J.Boil. Macromol. 107 (2018) 2611–2619.
[11] W. Wang, Y. Zou, Q. Li, R. Mao, X. Shao, D. Jin, D. Zheng, T. Zhao, H. Zhu, L. Zhang, L. Yang, X. Wu, Immunomodulatory effects of a polysaccharide purified from *Lepidium meyenii*, Walp. on macrophages, Process Biochem. 51 (2016) 542–553.
[12] X.L. Zhang, F. Ren, W. Huang, R.T. Ding, Q.S. Zhou, X.W. Liu, Anti-fatigue activity of extracts of stem bark from *Acanthopanax senticosus*, Molecules 16 (2010) 28–37.
[13] T.H. Pedersen, O.B. Nielsen, G.D. Lamb, D.G. Stephenson, Intracellular acidosis enhances the excitability of working muscle, Science 305 (2004) 1144–1147.
[14] A.D.H. Smith, S.L. Coakley, M.D. Ward, A.W. Macfarlane, P.S. Friedmann, N.P. Walsh, Exercise-induced stress inhibits both the induction and elicitation phases of in vivo T-cell-mediated immune responses in humans, Brain Behav. Immun. 25 (2011) 1136–1142.
[15] K. Azizbeigi, S.R. Stannard, S. Atashak, M.M. Haghighi, Antioxidant enzymes and oxidative stress adaptation to exercise training: comparison of endurance, resistance, and concurrent training in untrained males, J. Exerc. Sci. Fit. 12 (2014) 1–6.
[16] C. Xu, J. Lv, Y.M. Lo, S.W. Cui, X. Hu, M. Fan, Effects of oat β glucan on endurance exercise and its anti-fatigue properties in trained rats, Carbohydr. Polym. 92 (2013) 1159–1165.
[17] W. Tang, L. Jin, L. Xie, J. Huang, N. Wang, B. Chu, X. Dai, Y. Liu, R. Wang, Y. Zhang, Structural characterization and antifatigue effect in vivo of Maca (*Lepidium meyenii* Walp) polysaccharide, J. Food Sci. 82 (2017) 757–764.
[18] M. Zhang, G. Wang, F. Lai, Structural characterization and immunomodulatory activity of a novel polysaccharide from *Lepidium meyenii*, J. Agric. Food Chem. 64 (2016) 1921–1931.
[19] Q. Luo, Q. Sun, L. Wu, Z. Yang, Structural characterization of an immunoregulatory polysaccharide from the fruiting bodies of *Lepista sordida*, Carbohydr. Polym. 88 (2012) 820–824.

[20] H. Wang, G. Li, W. Zhang, C. Han, X. Xu, Y.P. Li, The protective effect of *Agaricus blazei* Murrill, submerged culture using the optimized medium composition, on alcohol-induced liver injury, Biomed. Res. Int. 10 (2014), 573978. .
[21] C.H. Lu, N.J. Engelmann, M.A. Lila, J.W. Erdman Jr., Optimization of lycopene extraction from tomato cell suspension culture by response surface methodology, J. Agric. Food Chem. 56 (2008) 7710–7714.
[22] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars, Anal. Chem. 28 (1956) 350–356.
[23] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[24] K. Zhu, Y. Zhang, S. Nie, F. Xu, S. He, D. Gong, et al., Physicochemical properties and in vitro antioxidant activities of polysaccharide from *Artocarpus heterophyllus* Lam. pulp, Carbohydr. Polym. 155 (2017) 354–361.
[25] V. Samavati, Polysaccharide extraction from *Abelmoschus esculentus*: optimization by response surface methodology, Carbohydr. Polym. 95 (2013) 588–597.
[26] G.J. Chen, S.Q. Zhang, C.X. Ran, L.S. Wang, J.Q. Kan, Extraction, characterization and antioxidant activity of water-soluble polysaccharides from *Tuber huidongens*e, Int. J. Biol. Macromol. 91 (2016) 431–442.
[27] S. Zha, Q. Zhao, J. Chen, L. Wang, G. Zhang, H. Zhang, B. Zhao, Extraction, purification and antioxidant activities of the polysaccharides from maca (*Lepidium meyenii*), Carbohydr. Polym. 111 (2014) 584–587.
[28] W.C. Huang, W.C. Chiu, H.L. Chuang, D.W. Tang, Z.M. Lee, L. Wei, F.A. Chen, C.C. Huang, Effect of curcumin supplementation on physiological fatigue and physical performance in mice, Nutrients 7 (2015) 905–921.
[29] B. Qi, L. Liu, H. Zhang, G. Zhou, S. Wang, X. Duan, X. Bai, S. Wang, D. Zhao, Anti-fatigue effects of proteins isolated from Panax quinquefolium, J. Ethnopharmacol. 153 (2014) 430–434.
[30] J.F. Ding, Y.Y. Li, J.J. Xu, X.R. Su, X. Gao, F.P. Yue, Study on effect of jellyfishcollagen hydrolysate on anti-fatigue and anti-oxidation, Food Hydrocoll. 25 (2011) 1350–1353.
[31] S.P. Cairns, Lactic acid and exercise performance, Sports Med. 36 (2006) 279–291.
[32] J. Wang, C. Sun, Y. Zheng, H. Pan, Y. Zhou, Y. Fan, The effective mechanism of the polysaccharides from Panax ginseng on chronic fatigue syndrome, Arch. Pharm. Res. 37 (4) (2014) 530–538.