International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

Maca (*Lepidium meyenii*) as a source of macamides and polysaccharide in combating of oxidative stress and damage in human erythrocytes

Lianzhu Lin,[1,2]* Jiayi Huang,[1] Dongxiao Sun-Waterhouse,[1,2] Mouming Zhao,[1,2,3] Kun Zhao[1,2] & Junjie Que[2]

1 School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China
2 Guangdong Food Green Processing and Nutrition Regulation Technologies Research Center, Guangzhou 510640, China
3 Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology & Business University, Beijing 100048, China

(Received 3 May 2017; Accepted in revised form 8 August 2017)

Summary Maca is known to be rich in macamides. The objective of this study was to analyse macamide profiles of macas with different colours cultivated in Yunnan, China, and commercial Peru maca by ultra-high-performance liquid chromatography–tandem mass spectrometry. The antioxidant activities of water, citric acid and alkali-extracted polysaccharides from Yunnan and Peru macas were evaluated via DPPH radical scavenging activity, oxygen radical absorbance capacity and erythrocyte haemolysis inhibition effect assays. Six major macamides present in Peru maca were also found dominant in Yunnan maca at comparable concentrations. Maca polysaccharide effectively scavenging DPPH and peroxyl radicals protected the erythrocytes against H_2O_2-induced haemolysis by inhibition of malondialdehyde (MDA) generation. Citric acid extraction was found the most suitable preparation method for generating heteropolysaccharide with strong antioxidant activity. Black maca cultivated in Yunnan possessing the most abundant macamides and polysaccharide with strong antioxidant activity could be developed as functional foods.

Keywords Extraction method, human erythrocyte, maca, macamide, oxidative stress, polysaccharide.

Introduction

Maca, a native plant in the central Andean region, possesses high carbohydrate, protein and unsaturated fatty acid contents (Clément *et al.*, 2010). It was found to be effective for treating arthritis, preserving cognitive function in middle-aged mice, improving memory, regulating hormone secretion and enhancing fertility (Gonzales *et al.*, 2006; Rubio *et al.*, 2007; Efthimiou & Kukar, 2010; Uchiyama *et al.*, 2014; Guo *et al.*, 2016; Ohta *et al.*, 2016). New areas for maca cultivation have been developed in other high-altitude regions including Yunnan province of China. The macamides are a distinct class of secondary metabolites, which have been identified as major characteristic compounds contributing to the efficacy of maca (McCollom *et al.*, 2005; Zhou *et al.*, 2017). Maca polysaccharide exhibited strong radical scavenging, antifatigue, immunomodulatory and hepatoprotective activities (Zha *et al.*, 2014; Zhang *et al.*, 2016, 2017a,b; Tang *et al.*, 2017). The use of citric acid to extract pectin is an efficient and eco-friendly extraction method (Oliveiral *et al.*, 2016). Citric acid extraction showed a positive influence on the antioxidant capacity of *Laminaria japonica* polysaccharide (Lu *et al.*, 2013).

Oxidative stress has been implicated in a number of human diseases as well as in the ageing process (Valko *et al.*, 2007). Erythrocytes are particularly susceptible to oxidation damage (Manna *et al.*, 1999). The H_2O_2-induced erythrocyte haemolysis visually revealed the oxidative damage to erythrocytes. However, there is little information on the protective effect of maca polysaccharide on oxidative stress-damaged human erythrocytes. In this study, the macamide profiles of Yunnan and Peru macas were compared. DPPH radical scavenging activities, oxygen radical absorbance capacities and protective effects against oxidative damage in human erythrocytes for all the water, citric acid and alkali-extracted polysaccharides were determined.

*Correspondent: Fax: +86 20 87113914; e-mail: felzlin@scut.edu.cn

doi:10.1111/ijfs.13586

Materials and methods

Samples

Black, violet and white macas collected from Binchuan County, Yunnan province, China, on 10 December 2014 were cleanly washed, dried in an oven at 45 °C and ground into fine powder. Peru maca (white maca) root powder was obtained from Mg Natura Peru Sac Company on 24 November 2014. All the samples were stored at 4 °C.

Chemicals

2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, Trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu Reagent, arabinose, glucose, galactose, mannose, galacturonic acid and glucuronic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The kit for MDA equivalent level was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). HPLC grade acetonitrile was purchased from Fisher (Pittsburgh, PA, USA). All other chemicals were of analytical grade.

UHPLC-MS/MS analysis of macamide profiles

Each maca powder (2 g) was subjected to ultrasound-assisted extraction (800 W) at 60 °C using 20 mL of ethanol for 60 min and centrifuged at 2000 ***g*** for 10 min. The supernatant was collected and stored at 4 °C till use. The UHPLC-MS/MS system was coupled with an electrospray ionisation (ESI) source in the positive ion mode. Separation of macamides was performed through an Agilent Zorbax Eclipse XDB UHPLC column (2.1 mm × 50 mm, 1.8 μm). The solvent system consisted of (A) water containing 0.005% trifluoroacetic acid and (B) acetonitrile using a gradient of 30:70 to 0:100 (A:B) in 8 min, followed by 100% B for 7 min. The flow rate was set at 0.8 mL min^{-1}. The major parameters for the MS/MS analysis were as follows: high-voltage capillary, 3500 V; scan range (m/z), 50–500.

Extraction of maca polysaccharide

Each maca powder (200 g) was refluxed for 3 h using 95% ethanol (2 L) and centrifuged at 2000 ***g*** for 10 min. The residue was extracted twice and dried at 50 °C for 10 h. The dried residue (150 g) of each maca mixed with 1.5 L of distilled water and treated with amylase at 55 °C for 8 h was equally divided into three groups. Group one was refluxed for 2 h and then centrifuged. The supernatant was collected, concentrated and mixed with cold 95% ethanol (4 °C, ethanol final concentration, 80%). The precipitate was collected, deproteinised using Sevag's method in triplicate, re-dissolved in distilled water and freeze-dried to obtain water-extracted polysaccharide. Group two treated with citric acid to reach pH 2.0 was refluxed for 2 h and then centrifuged. The supernatant was collected, adjusted to pH 7.0, concentrated and mixed with cold 95% ethanol. After deproteinising, polysaccharide was dialysed and freeze-dried to obtain citric acid-extracted polysaccharide. Group three treated with sodium hydroxide to reach pH 10.0 was refluxed for 2 h and then centrifuged. The supernatant was collected, adjusted to pH 7.0, concentrated and mixed with cold 95% ethanol. After deproteinising, polysaccharide was dialysed and freeze-dried to obtain alkali-extracted polysaccharide.

DPPH radical scavenging activity assay

DPPH radical scavenging activity was measured following our previously published method (Zhao *et al.*, 2014). Briefly, 2 mL of sample was mixed with 2 mL of 0.2 mM DPPH solution and left to stand for 30 min in dark. The absorbance was measured at 517 nm. Ethanol was used as a negative control. Trolox was used as a positive control. The absorbance of the blank was obtained by replacing DPPH with ethanol.

$$\begin{aligned}&\text{DPPH radical scavenging activity}\,(\%)\\&\quad=[1-(\text{Absorbance}_{\text{sample}}\\&\qquad-\text{Absorbance}_{\text{blank}})/\text{Absorbance}_{\text{negative control}}]\\&\qquad\times 100,\end{aligned}$$

DPPH value (μmol Trolox equivalents g^{-1}) referring to the required Trolox content (μmol) when DPPH radical scavenging activity of Trolox is equal to 1 g of maca polysaccharide was calculated using the regression equation between Trolox concentration and DPPH radical scavenging activity.

Oxygen radical absorbance capacity assay

The ORAC assay was conducted following our previously published method (Zhao *et al.*, 2014). Each sample (25 μL) was added in a well of a 96-well microplate, and then, 75 μL of fluorescein sodium salt (0.159 μM) was added. The mixture was pre-incubated for 10 min at 37 °C. Finally, 100 μL of AAPH (38.25 mM) was added. The fluorescence was measured every 60 s for 4200 s. The reaction was carried out at 37 °C. All the above measurements were performed on a plate reader (Thermo Fisher Scientific, Thermo Electron Co., Waltham, MA, USA) with an excitation wavelength of 485 nm and an emission wavelength of

530 nm. Phosphate buffer was used as a negative control. Trolox was used as a positive control.

$$\text{AUC (the net area under curve)} = 0.5(f_0 + f_n) + (f_1 + f_2 + \cdots + f_i + \cdots + f_{n-1}),$$

where f_0 is the initial fluorescence and f_i is the fluorescence reading at any time.

$$\text{Net AUC} = \text{AUC}_{\text{sample}} - \text{AUC}_{\text{negative control}}.$$

Oxygen radical absorbance capacity value (μmol Trolox equivalents g^{-1}) referring to the required Trolox content (μmol) when oxygen radical absorbance capacity of Trolox is equal to 1 g of maca polysaccharide was calculated using the regression equation between Trolox concentration and net AUC.

Erythrocyte haemolysis assay

The fresh blood was obtained from sixteen volunteers via venipuncture and collected in heparinised tubes. Erythrocytes were isolated by centrifugation at 1500 ***g*** for 10 min and washed 4 times with phosphate buffer saline (PBS, pH 7.4, containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4) and then re-suspended using PBS to obtain a 2% erythrocytes suspension. The antihaemolysis activity was determined by the method of Manna *et al.* (1999). Briefly, 200 μL of erythrocytes suspension was pre-incubated with 10 μL of sample at 37 °C for 30 min. Then, 200 μL of 400 mM H_2O_2 was added. The mixture was incubated at 37 °C for 1 h. The erythrocytes pretreated with PBS and then treated with H_2O_2 were used as negative control. After incubation, a batch of the reaction mixture (50 μL) was diluted with 250 μL of PBS, centrifuged at 2500 ***g*** for 10 min and measured at 412 nm (A). Another batch of the reaction mixture (50 μL) was treated with 250 μL of distilled water, centrifuged and measured at 412 nm (A_0).

$$\text{Haemolysis ratio}\,(\%) = (A/A_0) * 100\%.$$

The erythrocytes pretreated with maca polysaccharide and then treated with H_2O_2 were washed with PBS in triplicate and then lysed with distilled water. The mixture was centrifuged at 8000 ***g*** for 10 min at 4 °C. MDA equivalent level was determined using a kit.

Monosaccharide composition determination

The monosaccharide composition was performed by the method of Cheng *et al.* (2014). Maca polysaccharide (20 mg) was hydrolysed in 4 mL of 2 M trifluoroacetic acid at 110 °C for 4 h and centrifuged at 10 000 ***g*** for 5 min. The supernatant (400 μL) was evaporated until dry. The dried residue mixed with 0.6 M NaOH (100 μL) and 0.5 M 1-Phenyl-3-methyl-5-pyrazolone (PMP, 100 μL) was incubated at 70 °C for 100 min, before addition of 0.3 M HCl (100 μL) and chloroform. Monosaccharide standards were also subjected to derivatisation. The resultant aqueous layer was collected. The volume of the mixture was brought up to 2 mL with distilled water and filtered through a 0.45-μm membrane prior to HPLC analysis. Separation was conducted by isocratic elution with the mobile phase consisting of (A) phosphate buffer (20 mM, pH 6.7) and (B) acetonitrile (A:B = 83:17) at 1.0 mL min^{-1}. The wavelength for UV detection was 250 nm.

Statistical analysis

All the determinations were performed in triplicate. The final results were expressed as means ± standard deviations (SD). The significant differences between the means of parameters were calculated by Duncan's multiple-range test ($P < 0.05$) using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Identification of macamides in Yunnan and Peru macas

The total ion chromatograms of the ethanolic extracts of different macas are shown in Fig. 1. The UHPLC profiles of the different maca ethanol extracts differed mainly in the species appeared before the first 6-min retention time and the concentrations of individual compounds (1–6). Based on the MS^n data (Table 1), six compounds were identified as macamides: *n*-benzyl-(9Z,12Z,15Z)-octadecatrienamide (1), *n*-(3-methoxybenzyl)-(9Z,12Z)-octadecadienamide (2), *n*-benzyl-(9Z,12Z)-octadecadienamide (3), *n*-(3-methoxybenzyl)-hexadecanamide (4), *n*-benzyl-hexadecanamide (5) and *n*-benzyl-(9Z)-octadecenamide (6). The peaks with $[M + H]^+$ 368.2972 and 370.3135 suggested the formation of compounds 1 and 3 from linolenic acid, respectively. The peaks with $[M + H]^+$ 372.3289 suggested the formation of compound 6 from oleic acid. The main fragments derived from compound 5 contained a saturated alkyl-moiety, which matched the fragmentation patterns of capsaicinoids (Reilly *et al.*, 2003). The position of the methoxy substituent on the aromatic ring possibly resembled the compound of *n*-(3-methoxybenzyl)-hexadecanamide reported by Zhao *et al.* (2005). Thereby, the compounds 2 and 4 with $[M + H]^+$ 400.3264 and 376.3261 were possibly the 3-methoxy-derivatives of compounds 3 and 5, respectively.

n-Benzylhexadecanamide was the most abundant macamide in Peru maca. *n*-Benzyl-(9Z,12Z)-octadecadienamide

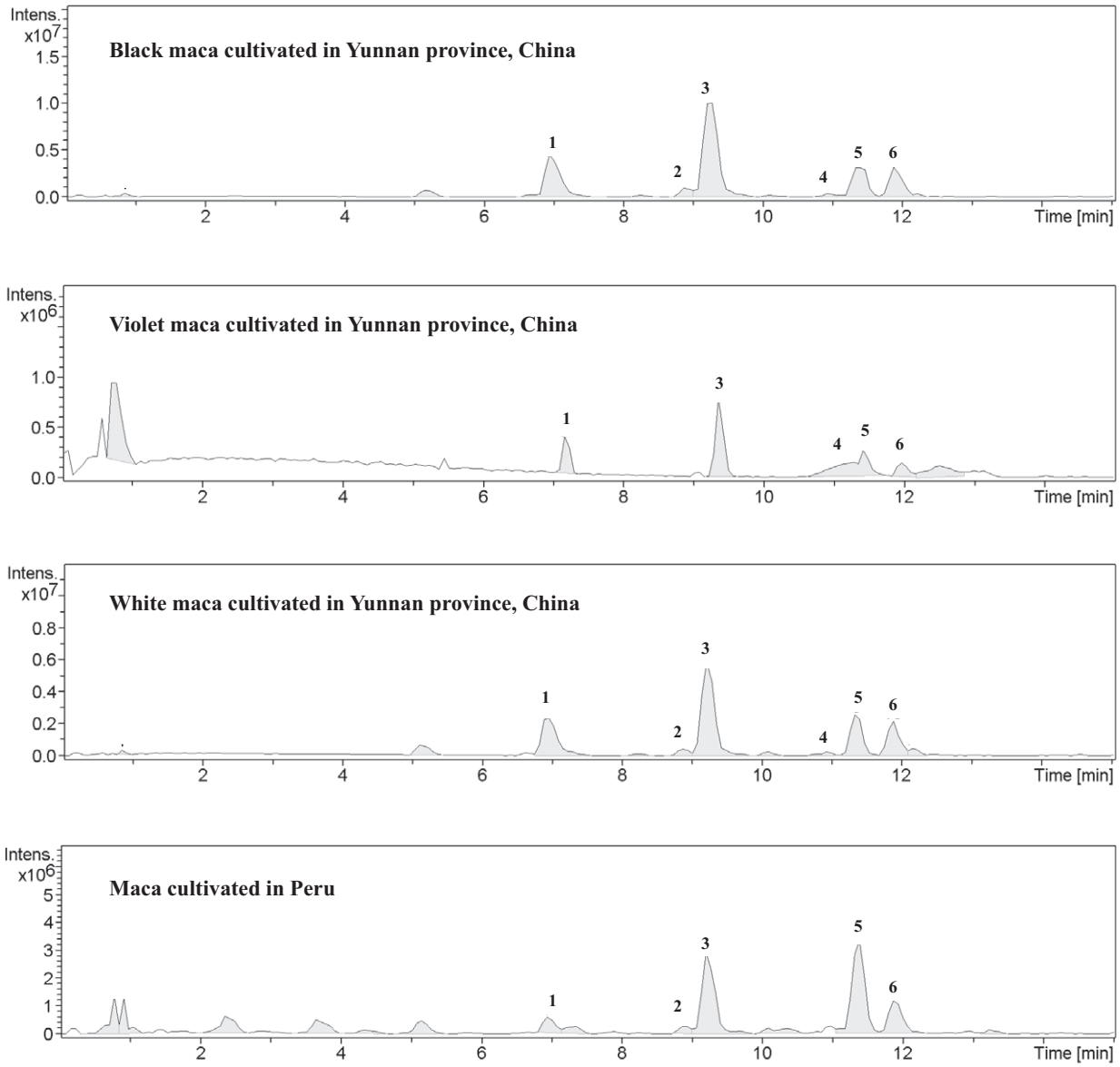

Figure 1 Total ion chromatograms of the ethanolic extracts of Yunnan and Peru macas.

was the compound with the highest concentration in Yunnan maca and the second highest concentration in Peru maca. The total amount of macamides in the obtained ethanolic extracts decreased in the order of black maca > white maca > Peru maca > violet maca. According to Pan *et al.* (2016), the highest peak area of macamides was found for black maca, which was consistent with our study. Black maca appeared to have more beneficial effect on sperm counts and epididymal sperm motility than the red or yellow maca (Gonzales *et al.*, 2006). The high total amount of macamides in black maca would likely explain the different bioactivities.

Antioxidant activity of maca polysaccharide

Antioxidant activity of maca polysaccharide was determined by analysing DPPH radical scavenging activity, oxygen radical absorbance capacity and erythrocyte haemolysis inhibition effect. As shown in Fig. 2a, for the same type of maca, the citric acid-extracted polysaccharide had the strongest DPPH radical

Table 1 Identified macamides in Yunnan and Peru macas

Compound	Macamide	Molecular formula	MS $[M + H]^+$	MS/MS fragments	Peak area			
					Yunnan maca			
					Black maca	Violet maca	White maca	Peru maca
1	*n*-Benzyl-(9Z,12Z,15Z)-octadecatrienamide	$C_{25}NOH_{37}$	368.2972	204.1384, 91.0607	71 222 184	2 679 031	37 602 088	6 931 293
2	*n*-(3-Methoxybenzyl)-(9Z,12Z)-octadecadienamide	$C_{26}NO_2H_{41}$	400.3264	220.1346, 121.0670	11 793 099	–	4 990 732	3 170 820
3	*n*-Benzyl-(9Z,12Z)-octadecadienamide	$C_{25}NOH_{39}$	370.3135	218.1572	157 399 456	6 234 357	74 785 120	36 273 316
4	*n*-(3-Methoxybenzyl)-hexadecanamide	$C_{24}NO_2H_{41}$	376.3261	121.0673	3 413 111	5 908 568	2 750 025	–
5	*n*-Benzylhexadecanamide	$C_{23}NOH_{39}$	346.314	239.1291, 91.0551	50 783 648	1 629 090	30 972 324	43 252 984
6	*n*-Benzyl-(9Z)-octadecenamide	$C_{25}NOH_{41}$	372.3289	195.0281, 91.0609	44 774 068	3 184 028	26 375 004	16 435 791

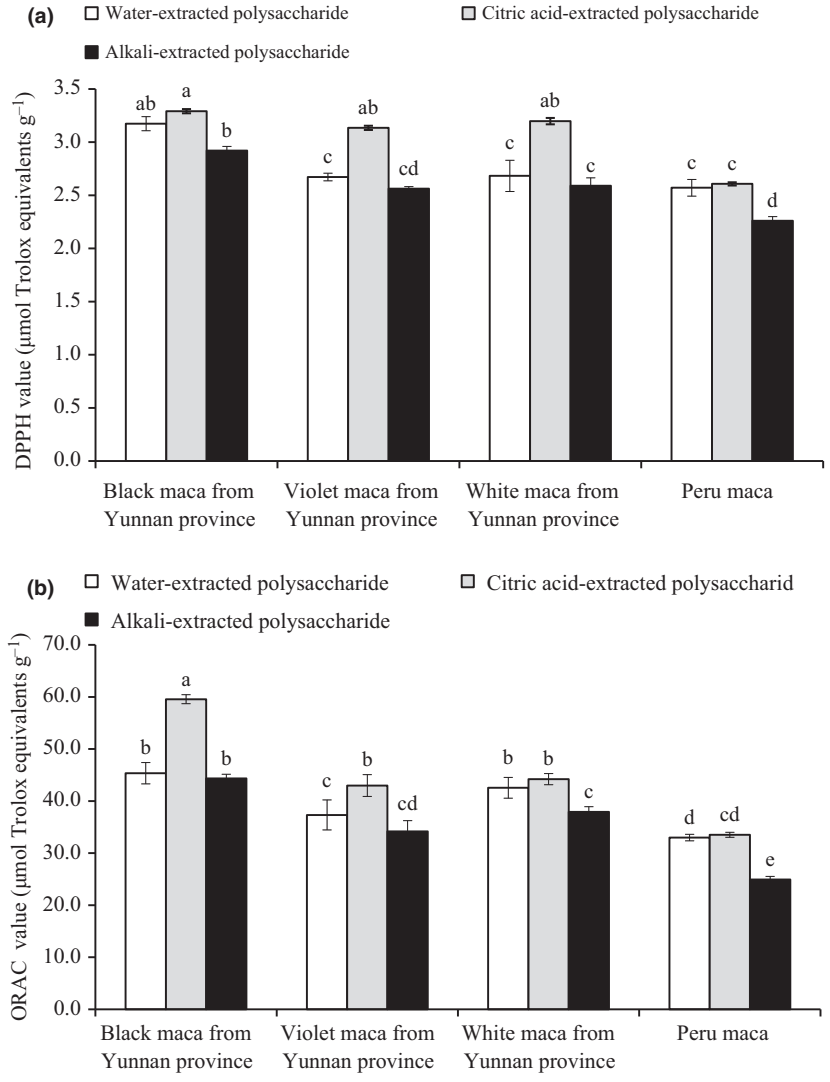

Figure 2 DPPH radical scavenging activities (a) and oxygen radical absorbance capacities (b) of water-, citric acid- and alkali-extracted polysaccharides. Different letters indicate $P < 0.05$.

scavenging activity (2.61–3.29 μmol Trolox equivalents g^{-1}). When the same extraction method was applied, black maca polysaccharide had the strongest DPPH radical scavenging activity (2.92–3.29 μmol Trolox equivalents g^{-1}). The changing pattern of the ORAC values for the different polysaccharides was similar to that detected in the DPPH assay (Fig. 2b). Citric acid extraction generally was the most efficient method to extract the antioxidative polysaccharides with high ORAC values (33.52–59.56 μmol Trolox equivalents g^{-1}). The citric acid-extracted polysaccharide of black maca possessed the highest DPPH value (3.29 μmol Trolox equivalents g^{-1}) and ORAC value (59.56 μmol Trolox equivalents g^{-1}). The effect of maca polysaccharide on DPPH and peroxyl radical scavenging might be due to hydroxyl groups which can donate hydrogen for binding with DPPH and peroxyl radicals to achieve the scavenging effect.

It was established that the erythrocyte haemolysis inhibiting activity reflects antioxidant activity (Liu *et al*., 2017). The citric acid-extracted polysaccharides from Yunnan maca and the alkali-extracted polysaccharides from black and white macas significantly protected the erythrocytes against H_2O_2-induced

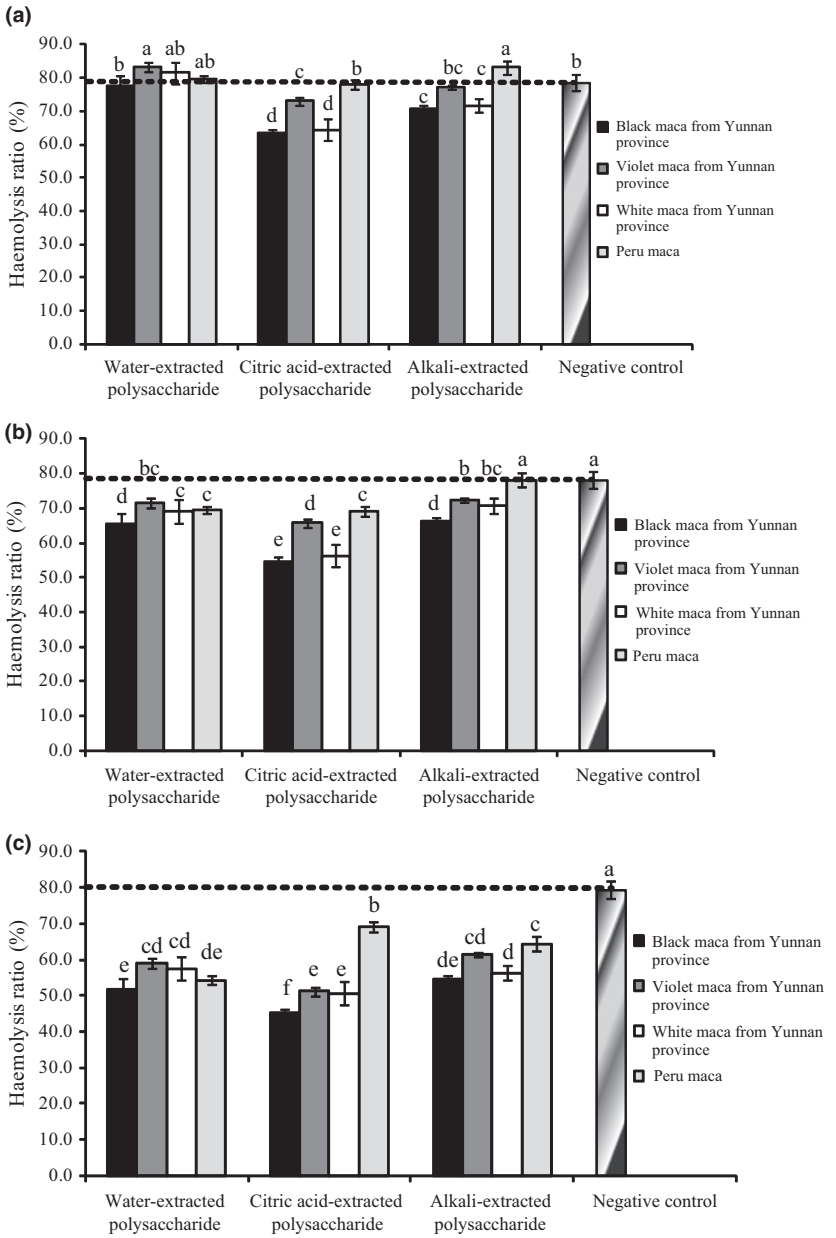

Figure 3 Haemolysis of erythrocytes treated with water-, citric acid- and alkali-extracted polysaccharides at 0.5 mg mL^{-1} (a), 1 mg mL^{-1} (b) and 2.5 mg mL^{-1} (c). Different letters indicate $P < 0.05$.

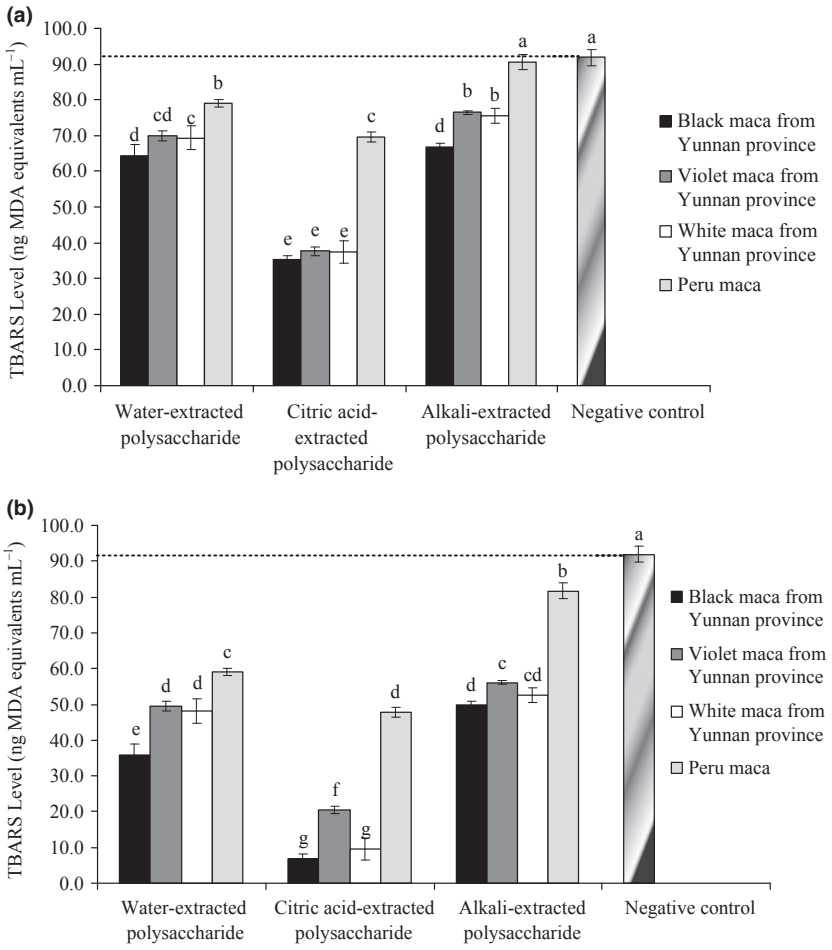

Figure 4 MDA equivalent generation of erythrocytes treated with water-, citric acid- and alkali-extracted polysaccharides at 1 mg mL^{-1} (a) and 2.5 mg mL^{-1} (b). Different letters indicate $P < 0.05$.

haemolysis at 0.5 mg mL^{-1} (Fig. 3a). At the concentration of 1.0 mg mL^{-1} (Fig. 3b), all polysaccharides from Yunnan maca protected oxidatively damaged erythrocytes. The citric acid-extracted polysaccharides from black and white macas exhibited the greatest protection effects. At the concentration of 2.5 mg mL^{-1} (Fig. 3c), all polysaccharides exerted significant protection on erythrocytes against H_2O_2-induced haemolysis. The citric acid-extracted polysaccharide from black maca provided the greatest protection effect. As erythrocytes lack the nuclei and mitochondria, its structural integrity is sensitive to changes of endogenous and exogenous reactive oxygen content. Any chemicals that can reduce oxidative stress could have ability to reduce erythrocyte injury in terms of oxidative stress hypothesis (An *et al.*, 2016). Maca polysaccharide effectively scavenging DPPH and peroxyl radicals protected the erythrocytes against H_2O_2-induced haemolysis. The antihaemolysis activity of polysaccharide mainly related to their active hydroxyl groups that can scavenge ROS (Zhang *et al.*, 2014).

MDA has been shown to cross-link erythrocyte phospholipids and proteins, which finally lead to haemolysis. The content of MDA in cells is regarded as an indirect index of oxidative damage (He *et al.*, 2009). All polysaccharides except for the alkali-extracted polysaccharide from Peru maca significantly decreased the TBARS levels at 1 mg mL^{-1} (Fig. 4a). In particular, the citric acid-extracted polysaccharides from Yunnan maca reduced the TBARS level by almost 2/3. As seen in Fig. 4b, maca polysaccharide effectively suppressed the formation of MDA at 2.5 mg mL^{-1}. In conclusion, in a cell-based antioxidant activity assay, maca polysaccharide (2.5 mg mL^{-1}) efficiently attenuated oxidative stress in human erythrocytes by inhibition of MDA and ROS generation.

Monosaccharide composition of citric acid-extracted polysaccharides

As seen in Table 2, the citric acid-extracted polysaccharides of Yunnan and Peru macas had the similar monosaccharide profiles. Glucose was identified as the

Table 2 Monosaccharide composition of citric acid-extracted polysaccharides of Yunnan and Peru macas

Monosaccharide composition (molar percentage)	Citric acid-extracted polysaccharide			
	Black maca	Violet maca	White maca	Peru maca
Mannose (%)	1.0	1.2	1.1	3.8
Glucose (%)	88.6	83.0	84.4	71.3
Galactose (%)	1.6	4.9	4.7	5.2
Arabinose (%)	6.3	8.3	8.0	17.3
Glucuronic acid (%)	1.2	1.0	1.5	1.3
Galacturonic acid (%)	1.1	1.7	0.3	1.1

main sugar (71.3–88.6%) in the citric acid-extracted polysaccharides with other monosaccharides including mannose (1.0–3.8%), galactose (1.6–5.2%), arabinose (6.3–17.3%), glucuronic acid (1.0–1.5%) and galacturonic acid (0.3–1.7%). The glucose content of Yunnan maca polysaccharide extracted by ultrasonic circulating technique was larger than rhamnose, galacturonic acid, galactose, xylose and arabinose (Zhang *et al*., 2017b). A polysaccharide from Peru maca mainly consisted of arabinose, mannose, glucose and galactose at approximate percentages of 20.9%, 4.5%, 71.9% and 2.7%, respectively (Zhang *et al*., 2017a). However, arabinose was the major monosaccharide in Yunnan maca (Zha *et al*., 2014). Tibet maca polysaccharide was composed of a high galacturonic acid content (Tang *et al*., 2017). The different monosaccharide analysis results may be attributed to the different cultivative conditions and the differences in extraction and analysis methods. It was already reported that not only the chemical composition but also the molecular mass and branching of the polysaccharides influenced their antioxidant capacities (Wang *et al*., 2010). It is worthy to mention that part of the detected antioxidant activities might also result from the residual phenolics (0.07–0.2%, data not shown) in the polysaccharides. Further investigation is under conduction to purify the citric acid-extracted polysaccharide and to evaluate its structure characteristics for illustration the structure–antioxidant activity relationship.

Conclusions

Black maca cultivated in Yunnan had the most abundant macamides and polysaccharide with strong antioxidant activity, compared with violet and white macas cultivated in Yunnan and commercial Peru maca. Citric acid extraction was found to be the most suitable preparation method for maca polysaccharide. Maca polysaccharide could be a good antioxidant and developed as functional foods.

Acknowledgments

Authors are grateful for the financial supports from Natural Science Foundation of Guangdong Province, China (Project No. 2015A030310494), National Natural Science Foundation of China (Project No. 31501424) and Guangzhou Science and Technology Plan Project (Project No. 20160402172 and 201710010060).

References

An, F., Wang, S.L., Yuan, D.H., Gong, Y.W. & Wang, S.H. (2016). Attenuation of oxidative stress of erythrocytes by plant-derived flavonoids, orientin and luteolin. *Evidence-Based Complementary and Alternative Medicine*, 3401269.

Cheng, B.H., Chan, J.Y.W., Chan, B.C.L. *et al*. (2014). Structural characterization and immunomodulatory effect of a polysaccharide HCP-2 from *Houttuynia cordata*. *Carbohydrate Polymers*, **103**, 244–249.

Clément, C., Grados, D.A.D., Avula, B. *et al*. (2010). Influence of colour type and previous cultivation on secondary metabolites in hypocotyls and leaves of maca (*Lepidium meyenii Walpers*). *Journal of the Science of Food and Agriculture*, **90**, 861–869.

Efthimiou, P. & Kukar, M. (2010). Complementary and alternative medicine use in rheumatoid arthritis: proposed mechanism of action and efficacy of commonly used modalities. *Rheumatology International*, **30**, 571–586.

Gonzales, C., Rubio, J., Gasco, M., Nieto, J., Yucra, S. & Gonzales, G.F. (2006). Effect of short-term and long-term treatments with three ecotypes of *Lepidium meyenii* (MACA) on spermatogenesis in rats. *Journal of Ethnopharmacology*, **103**, 448–454.

Guo, S.S., Gao, X.F., Gu, Y.R. *et al*. (2016). Preservation of cognitive function by *Lepidium meyenii* (maca) is associated with improvement of mitochondrial activity and upregulation of autophagy-related proteins in middle-aged mouse cortex. *Evidence-Based Complementary and Alternative Medicine*, 4394261.

He, J., Lin, J., Li, J., Zhang, J., Sun, X. & Zeng, C. (2009). Dual effects of *Ginkgo biloba* leaf extract on human red blood cells. *Basic & Clinical Pharmacology & Toxicology*, **104**, 138–144.

Liu, F., Wang, Y., Zhang, K., et al. (2017). A novel polysaccharide with antioxidant, HIV protease inhibiting and HIV integrase inhibiting activities from *Fomitiporia punctata* (P. karst.) murrill (Basidiomycota, hymenochaetales). *International Journal of Biological Macromolecules*, **97**, 339–347.

Lu, J.H., You, L.J., Lin, Z.Y., Zhao, M.M. & Cui, C. (2013). The antioxidant capacity of polysaccharide from *Laminaria japonica* by citric acid extraction. *International Journal of Food Science and Technology*, **48**, 1352–1358.

Manna, C., Galletti, P., Cucciolla, V., Montedoro, G. & Zappia, V. (1999). Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. *Journal of Nutritional Biochemistry*, **10**, 159–165.

McCollom, M.M., Villinski, J.R., McPhail, K.L., Craker, L.E. & Gafner, S. (2005). Analysis of macamides in samples of maca (*Lepidium meyenii*) by HPLC-UV-MS/MS. *Phytochemical Analysis*, **16**, 463–469.

Ohta, Y., Yoshida, K., Kamiya, S. *et al*. (2016). Feeding hydroalcoholic extract powder of *Lepidium meyenii* (maca) increases serum testosterone concentration and enhances steroidogenic ability of Leydig cells in male rats. *Andrologia*, **48**, 347–354.

Oliveiral, T.S., Rosa, M.F., Cavalcante, F.L. *et al*. (2016). Optimization of pectin extraction from banana peels with citric acid by using response surface methodology. *Food Chemistry*, **198**, 113–118.

Pan, Y., Zhang, J., Li, H., Wang, Y.Z. & Li, W.Y. (2016). Characteristic fingerprinting based on macamides for discrimination of maca (*Lepidium meyenii*) by LC/MS/MS and multivariate statistical analysis. *Journal of the Science of Food and Agriculture*, **96**, 4475–4483.

Reilly, C.A., Ehlhardt, W.J., Jackson, D.A. *et al*. (2003). Metabolism of capsaicin by cytochrome P450 produces novel dehydrogenated metabolites and decreases cytotoxicity to lung and liver cells. *Chemical Research in Toxicology*, **16**, 336–349.

Rubio, J., Dang, H.X., Gong, M.J., Liu, X.M., Chen, S.L. & Gonzales, G.F. (2007). Aqueous and hydroalcoholic extracts of Black Maca (*Lepidium meyenii*) improve scopolamine-induced memory impairment in mice. *Food and Chemical Toxicology*, **45**, 1882–1890.

Tang, W., Jin, L., Xie, L. *et al*. (2017). Structural characterization and antifatigue effect *in vivo* of maca (*Lepidium meyenii* Walp) polysaccharide. *Journal of Food Science*, **82**, 757–764.

Uchiyama, F., Jikyo, T., Takeda, R. & Ogata, M. (2014). *Lepidium meyenii* (Maca) enhances the serum levels of luteinising hormone in female rats. *Journal of Ethnopharmacology*, **151**, 897–902.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, **39**, 44–84.

Wang, J., Zhang, Q.B., Zhang, Z.S., Song, H.F. & Li, P.C. (2010). Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. *International Journal of Biological Macromolecules*, **46**, 6–12.

Zha, S.H., Zhao, Q.S., Chen, J.J. *et al*. (2014). Extraction, purification and antioxidant activities of the polysaccharides from maca (*Lepidium meyenii*). *Carbohydrate Polymers*, **111**, 584–587.

Zhang, C.M., Yu, S.H., Zhang, L.S., Zhao, Z.Y. & Dong, L.L. (2014). Effects of several acetylated chitooligosaccharides on antioxidation, antiglycation and NO generation in erythrocyte. *Bioorganic & Medicinal Chemistry Letters*, **24**, 4053–4057.

Zhang, M., Wang, G., Lai, F. & Wu, H. (2016). Structural characterization and immunomodulatory activity of a novel polysaccharide from *Lepidium meyenii*. *Journal of Agricultural and Food Chemistry*, **64**, 1921–1931.

Zhang, M., Wu, W., Ren, Y. *et al*. (2017a). Structural characterization of a novel polysaccharide from *Lepidium meyenii* (Maca) and analysis of its regulatory function in macrophage polarization *in vitro*. *Journal of Agricultural and Food Chemistry*, **65**, 1146–1157.

Zhang, L., Zhao, Q., Wang, L., Zhao, M. & Zhao, B. (2017b). Protective effect of polysaccharide from maca (*Lepidium meyenii*) on Hep-G2 cells and alcoholic liver oxidative injury in mice. *International Journal of Biological Macromolecules*, **99**, 63–70.

Zhao, J., Muhammad, I., Dunbar, D.C., Mustafa, J. & Khan, I.A. (2005). New alkamides from Maca (*Lepidium meyenii*). *Journal of Agricultural and Food Chemistry*, **53**, 690–693.

Zhao, M., Zhu, D., Sun-Waterhouse, D. *et al*. (2014). *In vitro* and *in vivo* studies on adlay-derived seed extracts: phenolic profiles, antioxidant activities, serum uric acid suppression, and xanthine oxidase inhibitory effects. *Journal of Agricultural and Food Chemistry*, **62**, 7771–7778.

Zhou, Y.Y., Li, P., Brantner, A. *et al*. (2017). Chemical profiling analysis of Maca using UHPLC-ESI-Orbitrap MS coupled with UHPLC-ESI-QqQ MS and the neuroprotective study on its active ingredients. *Scientific Reports*, **7**, 44660.