Alkali extraction and in vitro antioxidant activity of *Monascus* mycelium polysaccharides

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Revised: 1 November 2011 / Accepted: 13 January 2012 / Published online: 18 February 2012
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Abstract In the present work, alkali extraction technology was used to optimize the extraction of *Monascus* mycelium polysaccharides for the first time. The extracting parameters of alkali extracted *Monascus* mycelium polysaccharides were optimized by Box-Behnken design (BBD). The optimum conditions were extraction temperature 49 °C, alkali concentration 7%, solvent/material ratio 23:1 (ml/g) and extraction time 2.3 h with an enhanced yield of 10.1%, compared with the yield 4.76% of hot water extraction, indicating that alkali extraction is a more efficient way. In order to discuss the biological activity of alkali extracted polysaccharides, we compared the in vitro antioxidant activity of alkali extracted polysaccharides (AMP) with hot water extracted polysaccharides (HMP). The result showed that AMP have the similar capability of scavenging both superoxide radical and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical of HMP in vitro. Therefore, alkali extraction technology is not only a high-efficiency way to extract AMP, but also can retain the natural antioxidant activities of AMP, which can be used in pharmaceutical and food industries.

Keywords *Monascus* mycelium polysaccharides · Alkali extraction technology · Response surface methodology · Antioxidant activity

Introduction

Monascus is a traditional Chinese medicinal and edible fungus distributed in Fujian, Zhejiang, Taiwan and Guangxi

Province of China, which is a small filamentous saprophytic fungus and belongs to the *ascomycetes*. Its secondary metabolites such as *Monascus* pigment and γ -aminobutyric acid (GABA) had been widely studied for their chemical properties and biological activities (Zheng et al. 2010; Paola et al. 2011). The exopolysaccharides from *Monascus* had also attracted great attention in recent years, due to their various biological functions such as anti-tumor, anti-bacterial and antioxidant activity (Ding 2007; Cai et al. 2010). However, the alkali extraction technology for AMP and its antioxidant activities in vitro have not been studied.

Hot water extraction (HWE) is the most common method used for the extraction of polysaccharides. It should be noted that HWE of polysaccharides is associated with long extraction time and high temperature (Wang et al. 2010). In order to improve the yield of AMP, alkali extraction technology was firstly used to optimize the extracting parameters of AMP. Response surface methodology (RSM) is a collection of statistical and mathematical techniques that has been successfully used to determine the effects of several variables and optimize processes. RSM helps in determining the relationship between one or more measured responses and multiple input variables (Deepak et al. 2011), it can reduce the number of experimental trials and evaluate the interactions between multiple parameters. It is more effective and precise than many approaches (Guo et al. 2010). Box-Behnken design (BBD), one of RSM, was used to optimize the alkali extraction technology for AMP in present work. A BBD (4 factors and 3 levels) was applied to study the effects of extraction temperature, alkali concentration, solvent/material and extraction time on the yield of AMP.

A vast amount of circumstantial evidence implicates oxygen derived free radicals (especially superoxide and hydroxyl radical) as mediators of inflammation, shock, and ischemia/reperfusion injury (Song et al. 2010). Antioxidants have been widely used as food additives to protect against

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oxidative degradation by free radicals, and also help to prevent harmful reactive oxygen species in human body (Yim et al. 2011). Recently, some natural extracts such as polysaccharides extracted from fungus, bacteria and plant possess antioxidant activity (Xu et al. 2011a, b; Tian et al. 2011; Wang et al. 2011; Waseem et al. 2011). But little information is available about the antioxidant activity of the *Monascus* mycelium polysaccharides, and whether the antioxidant activities of polysaccharides were damaged by alkali extraction is still unclear. Therefore, the objective of this work was to compare the superoxide radical and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging abilities of the alkali extracted polysaccharides with hot water extracted polysaccharides in vitro.

Materials and methods

Microorganism and culture conditions

The *Monascus rubber* M-66 used in the experiment was isolated and purified from *Monascus*-fermented rice which collected from Zhejiang, China. Agar slants containing potato–dextrose–agar were inoculated with mycelia and incubated at 30 °C for 5 days, and then used as inoculum for seed culture. The seed culture was grown in 250 ml shake flasks for 2 days at initial pH 6.0, 30 °C, and 200 rpm with a medium containing glucose 50 g, yeast 1 g, peptone 5 g, KH₂PO₄ 1 g, MgSO₄·7H₂O 0.5 g, FeSO₄·7H₂O 0.01 g and distilled water 1,000 ml. The flask culture experiments were performed in 250 ml flasks containing 50 ml of the medium inoculating with 8% (v/v) of the seed culture (viable cell count 1.73×10^6 /ml). Submerged fermentation was carried out in the medium of following composition (g/L): glucose 50.0, peptone 10.0, KH₂PO₄ 8.0, MgSO₄·7H₂O 0.5, KCl 0.5, FeSO₄·7H₂O 0.01. All media were sterilized at 120 °C for 20 min. The fermentation cultivation was inoculated at 8% (v/v) of the above seed culture medium and kept at 30 °C and 200 rpm in 250 ml shake flasks for 96 h.

Alkali extraction of AMP and analytical methods

The mycelia were removed from the culture medium by centrifugation at 1,000 g for 20 min at 4 °C. The precipitate was washed repeated by distilled water and lyophilized. The dry mycelia were extracted with 95% ethanol for 24 h to remove impurities, pigments and small lipophilic molecules. The degreased mycelia were pulverized. After passing through a 60-mesh stainless steel sieve, the powder of *Monascus* mycelia was obtained. The powder of *Monascus* mycelium (1.0 g) was extracted at 40–60 °C, alkali concentration 3%–7%, solvent/material 10–30 (ml/g) and extraction time 1–3 h each time. After centrifugation and filtration,

the supernatants were mixed with four volumes of absolute ethanol, and then allowed to the precipitate overnight at 4 °C. The precipitate was collected by centrifuging at 12,000 g for 10 min, rewashed with absolute ethanol and centrifuged again as described above, then lyophilized. The washed precipitate was the *Monascus* mycelium polysaccharides (AMP). The AMP were dissolved in distilled water (50%, w/v). The solution was treated with Sevage agent (chloroform: n-butanol=4:1) and centrifuged to remove free protein, the protein content was estimated by Folin-Ciocalteu reagent using bovine serum albumin as standard (Lowry et al. 1951). And then, the supernatant was dialyzed using the dialysis bag (Mw cut-off 8,000–14,000 Da) against distilled water for 48 h at 4 °C with water replacement twice a day (Xu et al. 2011a, b). The retained fraction was recovered, concentrated under reduced pressure and lyophilized to obtain the polysaccharides (AMP-1). The sugar content was measured by phenol-sulfuric method using D-glucose as a standard (Dubois et al. 1956). The yield (%) of AMP was calculated as the sugar content of extraction/dried powder of *Monascus* mycelium weight.

Experimental design and statistical analysis

The aim of single factor tests was to optimize the main effective factors and the better ranges of parameters for RSM (Zhong et al. 2011). After determining the preliminary range of the extraction variables, a BBD with four independent variables (x_1 , extraction temperature; x_2 , alkali concentration; x_3 , solvent/material; x_4 extraction time) at three levels were performed. For statistical calculation, the variables were coded according to:

$$x_i = (X_i - X_0) / \Delta X_i \quad (1)$$

Where x_i is the independent variable coded value, X_i is the independent variable real value, X_0 is the independent variable real value on the centre point and ΔX_i is the step change value. The range of independent variables and their levels are presented in Table 1. The independent variables and their ranges were chosen based on preliminary experiment results. Table 2 shows the Box-Behnken design matrix of the experiment of

Table 1 Factors and levels of Box-Behnken test for optimization of the polysaccharides extraction from *Monascus*

Factors	Levels		
	−1	0	+1
X_1 : extraction temperature (°C)	40	50	60
X_2 : alkali concentration (%)	3	5	7
X_3 solvent/material (ml/g)	10	20	30
X_4 : extraction time (h)	1	2	3

Table 2 Box-Behnken design and results for optimization of the polysaccharides extraction from *Monascus*

Runs	X ₁	X ₂	X ₃	X ₄	Y (%)	
					Experimental	predicted
1	0	0	0	0	9.862	9.955
2	0	0	−1	−1	8.251	8.277
3	0	1	0	1	9.544	9.708
4	0	−1	0	1	9.178	8.964
5	0	1	−1	0	8.856	8.757
6	−1	1	0	0	9.563	9.381
7	0	−1	1	0	8.352	8.629
8	−1	0	1	0	9.175	8.996
9	0	0	1	1	9.036	9.003
10	1	1	0	0	8.885	8.766
11	−1	0	0	−1	9.123	9.317
12	0	1	0	−1	8.766	8.810
13	−1	0	0	1	7.952	7.991
14	1	0	−1	0	7.931	7.940
15	1	0	0	1	9.003	8.987
16	−1	0	−1	0	8.208	8.225
17	1	−1	0	0	8.023	8.197
18	0	1	1	0	9.483	9.674
19	−1	−1	0	0	9.138	9.250
20	0	−1	0	−1	9.189	8.855
21	0	0	1	−1	8.046	7.978
22	1	0	1	0	7.800	7.613
23	0	0	0	0	10.083	9.955
24	0	−1	−1	0	9.116	9.102
25	1	0	0	−1	6.515	6.654
26	0	0	0	0	9.921	9.955
27	0	0	−1	1	8.198	8.259

15 trials. All experiments were performed in triplicate and the averages of polysaccharides yield were taken as response.

Data from the BBD were analyzed by multiple regressions to fit the following quadratic polynomial model:

$$Y = \beta k_0 + \sum_{i=1}^4 \beta k_i X_i + \sum_{i=1}^4 \beta k_{ii} X_i^2 + \sum_{i < j=1}^4 \beta k_{ij} X_i X_j \quad (2)$$

Where Y is the response function, βk_0 is the centre point of the system, βk_i , βk_{ii} and βk_{ij} represent the coefficients of the linear, quadratic and interactive terms, respectively; x_i , x_{ii} and $x_i x_j$ represent the linear, quadratic and interactive terms of the coded independent variables, respectively (Yan et al. 2007). The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Lu et al. 2008). The quality of fit of

the polynomial model equation was expressed by the coefficient of determination R^2 . The Design Expert software trial version 7.0.0 (Stat-Ease, Minneapolis) was used to analyze the experimental data. The P-values of less than 0.05 were considered to be statistically significant.

Hot water extraction of HMP and analytical methods

The HMP yield of hot water extraction was measured according to the method of Dong (Dong et al. 2009): The powder of *Monascus* mycelium (1.0 g) was extracted thrice with each time for 110 min at 88.9 °C. After centrifugation and filtration, the supernatants were mixed with four volumes of absolute ethanol, and allowed to precipitate for overnight at 4 °C. The precipitate was collected by centrifuging at 12,000 g for 10 min, rewashed with absolute ethanol and centrifuged again as described above, lyophilized and got the HMP. The HMP were dissolved in distilled water (50%, w/v), then followed the method of alkali extraction, HMP-1 were obtained.

Assay for antioxidant activities

Superoxide anion scavenging assay

The superoxide anion-scavenging activity was measured following the method of Qi et al. (2006). Briefly, 1 ml of various concentrations of sample was mixed with 1 ml of 0.1 M phosphate buffer (pH 7.4) containing 150 μM NBT, 60 μM PMS and 468 μM NADH. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm. The ability to scavenge superoxide radical anion was expressed with following equation:

$$\text{Scavenging effect(\%)} = (1 - A_{\text{sample 560nm}}/A_{\text{control 560nm}}) \times 100\%$$

DPPH radical scavenging assay

The DPPH scavenging activity of AMP was measured according to the method of Liu and Zhao (2006). The reaction mixture contained 2 ml of 95% absolute ethanol, 0.1 μmol/l DPPH and 2 ml of AMP (0.5–5 mg/ml). The solution was incubated at 25 °C for 15 min, and the absorbance of AMP was determined at 517 nm. The antioxidant activity of AMP was evaluated according to the following formula:

$$\text{Scavenging rate(\%)} = (1 - A/A_0) \times 100\%$$

Where A was absorbance of AMP and A_0 was the absorbance of the DPPH solution.

Results and discussion

Effect of temperature on the yield of AMP by alkali extraction

Effect of different extraction temperature (30, 40, 50, 60, and 70 °C) on the yield of AMP is shown in Fig. 1a. Results' indicated that when the other reaction conditions were set as follows: alkali concentration 5%, solvent/material 20 ml/g, and extraction time 2 h. Figure 1a Results' indicated that the yield of AMP increased with the increase in extraction temperature and reached the peak value at 50 °C, after this point the yield of AMP inclined.

Effect of alkali concentration on the yield of AMP by alkali extraction

Effect of different alkali concentration (1%, 3%, 5%, 7%, and 9%) on the yield of AMP was seen in Fig. 1b, when the other reaction conditions were set as follows: extraction temperature 50 °C, solvent/material 20 ml/g, and extraction time 2 h. Figure 1b indicated that the yield of AMP reached the peak value when the alkali concentration was 5%. After this point, the yield of AMP had a slightly decline trends with the alkali concentration increasing.

Effect of solvent/material (10–50 ml/g) on the yield of AMP by alkali extraction

Effect of different solvent/material (10, 20, 30, 40, and 50 ml/g) on the yield of AMP was seen in Fig. 1c, when the other reaction conditions were set as follows: extraction temperature 50 °C, alkali concentration 5%, and extraction time 2 h. Figure 1c indicated that the yield of AMP had obvious increase within the solvent/material increasing and reached the peak value at the solvent/material 20 ml/g. Therefore, the appropriate solvent/material was 20 ml/g.

Effect of different extraction time on the yield of AMP by alkali extraction

Effect of different extraction time (1, 2, 3, 4, and 5 h) on the yield of AMP was seen in Fig. 1d, when the other reaction conditions were set as follows: extraction temperature 50 °C, alkali concentration 5%, and solvent/material 20 ml/g. Figure 1d indicated that the yield of AMP was enhanced to the peak value of 9.52% at the extraction time of 2 h, and then it appeared a descending trends when the extraction time

increasing. Therefore, 2 h was considered to be the optimal extraction time in this experiment.

Optimization of the procedure for alkali extraction

Statistical analysis and the model fitting

The values of responses (yield of AMP) at different experimental combination were given in Table 2. There were a total of 27 runs for optimizing the four individual parameters in the current BBD which was applied to the yield of AMP. Maximum yield of AMP (10.08%) was recorded under the experimental conditions of extraction temperature 50 °C, alkali concentration 5%, solvent/material 20 ml/g, and extraction time 2 h. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation:

$$Y = 9.96 - 0.42X_1 + 0.18X_2 + 0.11X_3 + 0.25X_4 + 0.11X_1X_2 - 0.27X_1X_3 + 0.91X_1X_4 + 0.35X_2X_3 + 0.20X_2X_4 + 0.26X_3X_4 - 0.95X_1^2 - 0.10X_2^2 - 0.81X_3^2 - 0.77X_4^2 \quad (3)$$

The model adequacy was checked by an F-test and the determination coefficient R^2 . The analysis of variance (Table 3) showed that the model was highly significant ($P < 0.001$) with F-value of 24.4. The p-value of 0.2121 (> 0.05) for lack of fit implies that it is not significant comparing to the pure error. The determination coefficient ($R^2 = 0.9661$) was showed by ANOVA of the quadratic regression model, indicating that only 3.4% of the total variations was not explained by the model. The value of the adjusted determination coefficient ($\text{Adj } R^2 = 0.9265$) also confirmed that the model was highly significant. Therefore, the model is found to be adequate for prediction within the range of experimental variables.

The regression coefficient values of Eq. (3) were listed in Table 3. The P values are used to check the significance of each coefficient, which in turn may indicate the pattern of the interaction between the variables (Zhang and Xue 2011). The smaller was the value of P , the more significant was the corresponding coefficient (Muralidhar et al. 2001). It can be seen from this table that the linear coefficients (x_1 , x_2 and x_4), a quadratic term coefficient (x_1^2 , x_3^2 and x_4^2) and cross product coefficients (x_1x_3 , x_1x_4 , x_2x_3 and x_3x_4) were significant, with very small P values ($P < 0.05$). The results of the study showed that extraction temperature was the most significant single parameter which influenced the yield of AMP, followed by extraction time, alkali concentration and

Fig. 1 Effect of different extraction parameters on the yield of Alkali extracted polysaccharides (AMP) (extraction temperature, alkali concentration, solvent/material and extraction time). Values are means of triplicate determinations ($n=3$) $\pm$ standard deviation

solvent/material. The full model fitted Eq. (3) was made three-dimensional and contour plots to predict the relationships between the independent and dependent variables (Sun et al. 2010).

Optimization of extraction conditions of AMP

The graphical representations of the regression Eq. (3), called the response surfaces and the contour plots were obtained using Design-Expert. The results of yield of AMP affected by extraction temperature, alkali concentration, solvent/material and extraction time are presented in Fig. 2. The 3D response surface and 2D contour plots provide a method to visualize the relationship between responses and experimental levels of each variable and the

type of interactions between two test variables. These types of plots show effects of two factors on the response at a time. In all the presented figures, the other two factors were kept at level zero (center value of the testing ranges). The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates that the interactions between the corresponding variables are negligible, while elliptical contour plot indicates that the interactions between the corresponding variables are significant (Muralidhar et al. 2001).

In Fig. 2a and b, when the 3-D response surface plot and the contour plot were developed for the yield of AMP with varying extraction temperature and solvent/material at fixed number of alkali concentration (0 level) and extraction time

Table 3 Statistical results of regression analysis for optimization of the polysaccharides extraction from *Monascus*

Source	Sum of squares	df	Mean square	F value	p-value Prob > F
Model	16.01	14	1.14	24.40	<0.0001
X ₁	2.09	1	2.09	4.49	<0.0001
X ₂	0.37	1	0.37	7.85	0.0160
X ₃	0.15	1	0.15	3.15	0.1011
X ₄	0.76	1	0.76	16.23	0.0017
X ₁ X ₂	0.048	1	0.048	1.02	0.3328
X ₁ X ₃	0.30	1	0.30	6.43	0.0261
X ₁ X ₄	3.35	1	3.35	71.41	<0.0001
X ₂ X ₃	0.48	1	0.48	10.32	0.0075
X ₂ X ₄	0.16	1	0.16	3.32	0.0934
X ₃ X ₄	0.27	1	0.27	5.80	0.0330
X ₁ ²	4.83	1	4.83	103.12	<0.0001
X ₂ ²	0.059	1	0.059	1.25	0.2853
X ₃ ²	3.50	1	3.50	74.65	<0.0001
X ₄ ²	3.13	1	3.13	66.83	<0.0001
Residual	0.56	12	0.047		
Lack of Fit	0.54	10	0.054	4.10	0.2121
Pure Error	0.026	2	0.013		
Cor Total	16.57	26			R ² =0.9661

(0 level), the yield of AMP increased with the increasing solvent/material, and reached the peak value rapidly at extraction temperature 49.42 °C, then dropped from 49.42 to 60 °C. In Fig. 2c and d, when the 3D response surface plot and the 2D contour plots were developed for the yield of AMP with varying extraction temperature and extraction time at fixed alkali concentration (0 level) and solvent/material, it was revealed that the maximum yield (10.08%) of AMP was achieved when the extraction time 2.32 h and extraction temperature 49.42 °C were used. The 3D response surface plot and 2D contour plots based on independent variables alkali concentration and solvent/material are shown in Fig. 2e and f, while extraction temperature and extraction time were kept at 0 levels. It can be seen that the yield of AMP had a slightly increase with the alkali concentration increasing, but with the solvent/material increasing, the yield of AMP reached the peak value rapidly at 23.44 ml/g, after this point the yield of AMP declined rapidly. The Fig. 2g and h showed the 3-D response surface plot and the contour plot at varying solvent/material and extraction time at fixed extraction temperature (0 level) and alkali concentration (0 level). It can be seen that maximum yield of AMP can be achieved when solvent/material and extraction time are 23.44 ml/g and 2.32 h, respectively.

By analyzing the plots, the predicted values (10.18%) of the tested variables for the yield of AMP, lied in the

following condition: extraction temperature 49.42 °C, alkali concentration 6.99%, solvent/material ratio 23.44 (ml/g) and extraction time 2.32 h. Taking account of the operating convenience, the optimal parameters were determined as following: extraction temperature 49 °C, alkali concentration 7%, solvent/material ratio 23:1 (ml/g) and extraction time 2.3 h. Under such conditions, the yield of AMP was about 10.1%, slightly less than that of the maximum predicted value.

Verification of predictive model

In order to validate the adequacy of the model equations (Eq. (3)), a verification experiment was carried out under the optimal conditions mentioned above. Under the optimal conditions, the model predicted a maximum response of 10.18%. A mean value of 10.1, obtained from real experiments, demonstrated the validation of the extraction model. The results indicate that the experimental values are in good agreement with the predicted one, and also suggest that the regression model is accurate and adequate for the extraction of AMP.

Result of hot water extraction of HMP

Under the optimal conditions of Dong et al. (2009) (Extraction time 110 min, extraction temperature 88.9 °C, extraction number of times 3), the yield of HMP reached 4.76%, which is lower than alkali extraction.

Antioxidant activities of AMP

Superoxide anion radical scavenging assay

As can be seen in Fig. 3a, the results indicated a concentration-dependent radical-scavenging activity at all tested concentrations of the samples. AMP and HMP had the similar capability of scavenging superoxide anion radical at the test concentration range. At the concentration of 2.25 mg/ml, the scavenging effect was 80.67% and 78.82 for AMP and HMP respectively. These results indicated that AMP can still retain higher superoxide anion radical scavenging activity with HMP.

DPPH radical scavenging assay

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds (Chen et al. 2008). The DPPH assay

Fig. 2 Response surface plots and contour plots showing the effects of variables (X₁: extraction temperature; X₂: alkali concentration; X₃: solvent/material; X₄: extraction time) on the response Y (the yield of Alkali extracted polysaccharides (AMP))

Fig. 3 Scavenging effects of the Alkali extracted polysaccharides (AMP) and Hot water extracted polysaccharides (HMP) on (a) superoxide anion radical and (b) DPPH radical in vitro ($n=3$). Values are means of triplicate determinations ($n=3$) $\pm$ standard deviation

involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) wherein a reduction in the DPPH concentration caused by the antioxidant decreases the optical absorbance of DPPH (Annegowda et al. 2011). Figure 3b describes the scavenging ability of AMP and HMP on DPPH radical. In the concentration ranged from 0.5 to 3 mg/ml, AMP had the similar capability of scavenging DPPH radical, and theirs scavenging

activities increased rapidly with concentration increasing. The highest scavenging activity of HMP and AMP was 40.64% and 38.73% at the concentration of 0.875 mg/ml, respectively. Sun et al. (2009) found that the scavenging ability on DPPH radical was 23.4% when *Ganoderma atrum* extracellular polysaccharides reached at 40 mg/ml. Therefore, this ability of HMP and AMP to scavenge DPPH radical may contribute to their significant antioxidant potential.

Main compositions and antioxidant activities of different treated *Monascus mycelium polysaccharides*

In order to identify the main antioxidant activities of ethanol extracts, the sugar and protein content of two different extracted polysaccharides were analyzed and their antioxidant activities were compared. As can be seen in Table 4, after deproteinization and dialysis, the sugar content of AMP and HMP was increased to 93.47% and 95.82%, the protein content of AMP and HMP was declined to 1.08% and 0.87%, but the antioxidant activities of AMP and HMP increased with the increase in sugar content. The results indicated that the main compositions of ethanol extracts were sugar and protein, but polysaccharides were main compositions of antioxidant activities.

Conclusion

In conclusion, response surface methodology using second-order regression for a four-factor-three-level Box-Behnken design was a successful tool for alkali extraction optimization of AMP. The optimal conditions (extraction temperature 49 °C, alkali concentration 7%, solvent/material ratio 23:1 (ml/g) and extraction time 2.3 h.) for the yield of AMP were estimated using the model equation. Under these conditions, the mean value of AMP was 10.1%, compared with the yield 4.76% of HMP, alkali extraction is a more efficient method. Antioxidant activities test in vitro shows that both HMP and AMP possess the similar scavenging effect of

Table 4 Superoxide anion radical and DPPH radical scavenging ability of different treated polysaccharides from *Monascus* at concentration of 1.5 mg/ml

	Sugar content (%)	Protein content (%)	Superoxide anion radical scavenging ability (%)	DPPH radical scavenging ability (%)
Alkali extracted polysaccharides (AMP)	82.43 $\pm$ 0.445	11.75 $\pm$ 1.108	51.66 $\pm$ 1.330	27.31 $\pm$ 0.721
AMP-1	93.47 $\pm$ 1.217	1.08 $\pm$ 0.345	54.36 $\pm$ 0.991	29.69 $\pm$ 1.107
Hot water extracted polysaccharides (HMP)	86.62 $\pm$ 0.773	9.51 $\pm$ 0.367	54.99 $\pm$ 0.721	28.82 $\pm$ 1.133
HMP-1	95.82 $\pm$ 0.474	0.87 $\pm$ 1.152	56.72 $\pm$ 0.268	30.74 $\pm$ 0.632

superoxide radical and DPPH radical. Therefore, the present study demonstrates that alkali extraction technology is not only a high-efficiency way to extract AMP, but also can retain the natural antioxidant activities of AMP.

Acknowledgements This work was supported by the Natural Science Foundation of China (NO.31070008) and Natural Science Foundation of Zhejiang Province (Grant Y3090343), Republic of China.

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