

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/301707275>

Ultrasonic-assisted enzymatic extraction and antioxidant activity of polysaccharides from *Setaria viridis*

Article in *Separation Science and Technology* · April 2016

DOI: 10.1080/01496395.2016.1178287

CITATIONS

0

READS

18

8 authors, including:



Shu Yuan

Sichuan Agricultural University

98 PUBLICATIONS 1,061 CITATIONS

[SEE PROFILE](#)



Yanger Chen

Umeå University

42 PUBLICATIONS 319 CITATIONS

[SEE PROFILE](#)



Ming Yuan

Sichuan Agricultural University

41 PUBLICATIONS 218 CITATIONS

[SEE PROFILE](#)

Ultrasonic-assisted enzymatic extraction and antioxidant activity of polysaccharides from *Setaria viridis*

Le Li^a, Xu Li^b, Chunbang Ding^b, Shu Yuan^c, Zhongwei Zhang^c, Yanger Chen^b, Chao Hu^b, and Ming Yuan^b

^aCollege of Science, Sichuan Agricultural University, Ya'an, Sichuan, China; ^bCollege of Life Science, Sichuan Agricultural University, Ya'an, Sichuan, China; ^cCollege of Environment, Sichuan Agricultural University, Chengdu, Sichuan, China

ABSTRACT

Ultrasonic-assisted enzymatic extraction (UEAE) and response surface methodology (RSM) were used to isolate polysaccharides from *Setaria viridis* (SVP). Optimal extraction conditions in the enzymatic hydrolysis process were: extraction duration, 68 min; extraction temperature, 51°C; ratio of enzyme to raw material, 1.6%; and ratio of liquid to raw material, 20 mL/g. Then, following ultrasonic treatment (180 W, 60°C, 60 min), the experimental yield was $8.94 \pm 0.38\%$. Crude SVP was purified by DEAE cellulose-52 chromatography and Sephadex G-100 chromatography, resulting in the isolation of three fractions (designated SVP-1, SVP-2 and SVP-3). These SVPs were mainly composed of glucose residue, and SVP-3 had a significantly higher uronic acid content than the other two fractions. Additionally, all fractions showed strong antioxidant activities *in vitro*.

ARTICLE HISTORY

Received 4 August 2015
Accepted 11 April 2016

KEYWORDS

Antioxidant activity; *Setaria viridis* polysaccharides; ultrasonic-assisted enzymatic extraction

Introduction

Under normal circumstances homeostasis is maintained through a balance between the production and clean-up of reactive oxygen species (ROS) in organisms.^[1] When the production of ROS is excessive, oxidative stress occurs. These uncontrolled ROS may result in extensive oxidative damage on macromolecules, such as protein, lipids and nucleic acid, which is associated with cancer, diabetes mellitus and neurodegenerative and inflammatory diseases.^[2,3] Thus, it is needed to discover and explore more antioxidants to relieve oxidative stress. Fortunately, polysaccharides derived from traditional Chinese herbs have the potential to be developed as natural antioxidants due to their strong antioxidant ability and low cytotoxicity.^[4] Recent high-profile reports indicated that polysaccharides from herbs possessed numerous biological activities, including antiviral,^[5] antitumor,^[6] immunomodulation^[7] and anti-oxidation.^[8]

Conventionally, polysaccharides were isolated from plants by hot water method (HWM). However, HWM is very time-consuming and requires high temperature.^[9] Enzyme-assisted extraction (EAE) could avoid high temperature but with relatively long extraction time and low extraction yield.^[10] In contrast, ultrasonic-assisted extraction (UAE), which is faster and requires less energy and less solvent, has been widely used for the extraction of target compounds from biological materials.^[11] Thus,

EAE coupled with UAE might be an effective and advisable method for extraction of polysaccharides.

Setaria viridis (L.) Beauv. belongs to the family of Gramineae, and is a traditional medicinal plant in China. Native to the temperate and subtropical regions of Eurasia, it is now widely distributed as an introduced species.^[12] In China, *S. viridis* was traditionally used to treat acute eczema, warts and corns, and to relieve abdominal distension, belch and diarrhoea.^[13–15] Recent studies showed that *S. viridis* is rich in polyphenols, tannins and sugars, and it exhibits powerful antioxidant, anti-inflammatory, anti-allergic and anti-pruritic properties.^[16–18]

Response surface methodology (RSM) can save time and labour, and reduce the number of experimental trials when interactions of multiple factors need to be evaluated.^[19] The Box–Behnken design (BBD), one of the most common models of RSM, is widely used to optimize extraction conditions.^[20] In the present study, polysaccharides were extracted from *S. viridis* using an ultrasonic-assisted enzymatic extraction (UEAE) method. In addition, BBD was used to optimize key extraction factors, including ratio of water to raw material, ratio of enzyme to raw material, extraction time and extraction temperature. Then, the crude polysaccharides of *S. viridis* were purified. The physicochemical and structural properties of purified fractions were determined, as well as *in vitro* antioxidant activities.

Materials and methods

Materials and reagents

The fresh aerial part of *S. viridis* was collected from Ya'an suburb, Sichuan province, China. Materials were washed thoroughly with water, dried at 50°C to achieve constant weight, pulverized in a powerful mill (FW177, Taisite Instrument Co., Ltd., Tianjin, China) and screened through a 30-mesh sieve. This powder was stored in a desiccator at room temperature.

Cellulase and pectase were purchased from Shanghai Ryon Biological Technology Co., Ltd. (Shanghai, China). Both DEAE cellulose-52 and Sephadex G-100 were purchased from Kayon Biological Technology Co., Ltd. (Shanghai, China). 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 1,10-phenanthroline, trichloroacetic acid (TCA) and ferrozine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of crude SVP

The *S. viridis* powder was refluxed with petroleum ether, acetone and ethyl alcohol, respectively, for 6 h. Then, 10 g of powder was hydrolysed with cellulase-pectase (7:3, pH 5.0) at a given temperature, time, ratio of enzyme to raw material and ratio of water to raw material. After hydrolysis, the extraction slurry was exposed to an ultrasonic wave (180 W) at 60°C for 60 min, and then centrifuged at 7000 g for 10 min. Supernatant was collected and concentrated to 20% of initial volume using a vacuum rotary evaporator at 60°C, and then precipitated with five volumes of anhydrous ethanol (4°C) overnight. After centrifugation, the precipitate was vacuum-dried at 60°C to a constant weight. The SVP yield (%) was calculated as follows:

$$\text{Yield (\%)} = \frac{A}{B} \times 100$$

where A and B were the weight of crude polysaccharides (g) and *S. viridis* powder (g), respectively.

Experimental design for extraction

Based on the single-factor test, a four-factor three-level BBD was used to optimize extraction conditions. Four key parameters during enzymatic hydrolysis processing stages, including extraction temperature (X1), ratio of water to raw material (X2), extraction time (X3) and ratio of enzyme to raw material (X4), were the independent (input) variables, whereas crude polysaccharides yield (Y) was the dependent (output) variable. The real values of each factor are shown in Table 1. To

predict the optimal value of each factor, a second-order polynomial equation was fitted to determine the relationship between factors and response values. For each response value, the significance was evaluated by analysis of variance (ANOVA). The accuracy and general ability of the polynomial model were evaluated by the coefficient R². In addition, validation experiments were carried out.

Separation and purification of SVP

Crude polysaccharides were extracted under optimized extraction conditions, and deproteinized with Sevag reagent (chloroform:butanol, 4:1). The obtained polysaccharides were redissolved in distilled water, filtered through a 0.45 µm membrane and loaded on a DEAE-52 cellulose column (2.6 × 60 cm). The stepwise elution was 0, 0.1, 0.3, 0.5 and 0.7 mol/L NaCl solutions at a flow rate of 0.6 mL/min. The elution solution (5 mL/tube) was collected with an automatic collector, and monitored by the phenol-sulphuric acid method at 490 nm. Three main elution peaks (SVP-1, SVP-2 and SVP-3) were individually collected, concentrated, dialyzed and lyophilized. Further purification was performed

Table 1. Experimental result of response surface methodology.

Run ^a	X1 ^b	X2 ^b	X3 ^b	X4 ^b	Yield(%) ^c
1	60	10	60	2	7.00
2	50	20	90	1	8.40
3	50	20	60	2	8.90
4	40	20	30	2	6.67
5	60	20	60	1	7.71
6	50	20	60	2	8.90
7	50	30	60	1	7.81
8	50	30	90	2	7.53
9	50	20	60	2	9.01
10	50	10	30	2	7.12
11	50	10	60	3	7.69
12	50	20	60	2	8.90
13	40	20	60	3	7.11
14	40	20	60	1	6.95
15	40	10	60	2	6.99
16	60	20	30	2	6.05
17	60	30	60	2	6.76
18	60	20	90	2	7.31
19	50	20	30	1	6.54
20	50	10	90	2	7.40
21	50	30	30	2	6.73
22	50	30	60	3	7.22
23	40	20	90	2	6.14
24	50	20	60	2	8.90
25	60	20	60	3	6.66
26	40	30	60	2	6.43
27	50	20	90	3	6.62
28	50	10	60	1	7.78
29	50	20	30	3	7.56

^aX1: extraction temperature (°C); X2: ratio of water to raw material; X3: extraction time (min); X4: ratio of enzyme to raw material.

^bThe other conditions were fixed at ratio of water to raw material 20 mL/g, extraction temperature 50 °C, extraction time 60 min and ratio of enzyme to raw material 2%.

^cMean of three replicate extractions, Relative Standard Deviation (RSD) < 3%.

with a Sephadex G-100 column (2.6 × 60 cm), and eluted with distilled water at a flow rate of 0.5 mL/min. The elution solution (5 mL/tube) was collected, concentrated, dialyzed and lyophilized.

Characterization of purified polysaccharides

Molecular weight determination

Average molecular weights of SVP-1, SVP-2 and SVP-3 were determined by high-performance size exclusion chromatography (HPSEC) at a flow rate of 0.6 mL/min.^[21] The calibration curve was made with 5 dextrans (T-500, T-200, T-100, T-50 and T-10).

Preliminary chemical composition analysis

Protein was determined by coomassie brilliant blue G250 staining method,^[22] taking bovine serum albumin (BSA) as standard. Total sugars were determined by the phenol-sulphuric acid method,^[23] taking glucose as standard. Uronic acid was measured by the vitriol-carbazole method,^[24] taking D-glucuronic acid as the standard.

Monosaccharide composition analysis

Monosaccharide compositions of SVP-1, SVP-2 and SVP-3 were analysed by GC-MS.^[20] Each sample (10 mg) was hydrolysed with trifluoroacetic acid at 110°C for 12 h in an ampoule bottle. Excessive acid was removed by vacuum evaporation with methanol. Hydrolysates were reduced by NaBH₄, and acetylated by acetic anhydride at 40°C for 3 h; then the solution was analysed by GC-MS with a Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The operation parameters of GC-MS were as follows: column temperature was increased from 120°C to 170°C at 10°C/min (holding for 3 min), then increased to 280°C at 5°C/min (holding for 15 min). Injection and detector temperatures were 220°C and 280°C, respectively. The carrier gas was helium at a flow rate of 1 mL/min.

Fourier-transform infrared spectroscopy (FTIR) assay

The FTIR spectrum of SVPs was recorded by a Fourier transform infrared spectrophotometer (FTIR-8400, Shimadzu, Tokyo, Japan). Samples were ground with KBr and pressed into a pellet for spectrometric analysis in the frequency range of 4000–500 cm⁻¹.

Antioxidant activity analysis

DPPH scavenging activity

The DPPH scavenging activity was determined by the spectrophotometric method.^[19] A total of 1 mL of sample solution (0–2.4 mg/mL) was added into 4.0 mL of DPPH solution (0.15 mmol/L in 90% ethanol). The mixture was

incubated for 30 min in the dark at room temperature. Absorbance was recorded at 517 nm and DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_o - A_s}{A_o - A_h} \times 100$$

where A_o is the absorbance of the mixture with distilled water; A_s is the absorbance of the mixture with sample; and A_h is absorbance of the sample without adding DPPH solution.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined by the spectrophotometric method.^[25] The reaction mixture (0.5 mL of 0–2.4 mg/mL samples, 1 mL of 0.75 mmol/L FeSO₄, 1 mL of 0.1% H₂O₂, 1 mL of 0.75 mmol/L 1,10-phenanthroline and 1 mL of 0.1 mol/L potassium phosphate buffer, pH 7.4) was incubated for 1 h at 37°C. The absorbance was recorded at 535 nm. The hydroxyl radical scavenging activity was calculated as follows:

$$\begin{aligned} \text{Hydroxyl radical scavenging activity (\%)} \\ = \frac{A_s - A_o}{A - A_o} \times 100 \end{aligned}$$

where A_s is the absorbance of the mixture with the sample; A_o is the absorbance of the mixture with distilled water; and A is the absorbance of the mixture with the sample, but hydrogen peroxide was replaced by distilled water.

Reducing power

The reducing power of samples was evaluated as the previous method with slight modifications.^[26] Briefly, 1 mL of sample solution (0–2.4 mg/mL) was mixed with 2.5 mL of 0.2 mol/L potassium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and then incubated at 50°C for 20 min. Thereafter, 2.5 mL of 10% TCA was added, and centrifuged for 10 min at 4000 g. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance at 700 nm was recorded as the reducing power.

Iron chelating capacity

The iron chelating capacity was measured by the spectrophotometric method.^[26] An aliquot (1 mL) of sample solution (0–2.4 mg/mL) was mixed with 3.7 mL of distilled water, 0.1 mL of 2 mmol/L FeCl₂ and 0.1 mL of 5 mmol/L ferrozine at room temperature. After 20 min, absorbance was measured at 562 nm. The iron chelating capacity was calculated as follows:

$$\text{Iron chelating capacity (\%)} = \frac{A_o - A_s}{A_o} \times 100$$

where A_0 is the absorbance of the mixture with distilled water and A_s is the absorbance of the mixture with samples.

Statistical analysis

Results were expressed as mean \pm SD with three repeats. All data were analysed by Origin pro8.5. One-way ANOVA was applied by Dunnett's test, and values of $p < 0.05$ were considered significant.

Results and discussion

Optimization of extraction conditions

Polynomial model fitting

The effects of four individual variables in the enzymatic hydrolysis process, including ratio of water to raw material (X1), extraction temperature (X2), ratio of enzyme to raw material (X3) and extraction time (X4), on the yield of SVP (Y), are shown in Table 1. Based on multiple regression analysis, response and test variables are expressed by the following second-order polynomial equation:

$$Y = 8.92 + 0.1X1 - 0.12X2 + 0.23X3 - 0.19X4 + 0.7X3X4 - 0.133X1^2 - 0.74X2^2 - 10.4X3^2 - 0.55X4^2$$

Coefficients and ANOVA analysis of the predicted model are shown in Table 2. This model is significant ($p < 0.01$), coefficient is high ($R^2 = 0.9939$), variation coefficient is low ($CV = 1.3\%$) and lack of fit is not significant ($p > 0.05$). Therefore, this fitting model is precise and reliable to predict SVP yield.

Table 2. Coefficients and ANOVA analysis of the fitting model.

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-Value
Model	21.39	14.00	1.53	164.26	< 0.0001**
X1	0.12	1.00	0.12	13.06	0.0028**
X2	0.19	1.00	0.19	20.15	0.0005**
X3	0.62	1.00	0.62	67.06	< 0.0001**
X4	0.45	1.00	0.45	48.75	< 0.0001**
X1X2	0.03	1.00	0.03	2.82	0.12
X1X3	0.80	1.00	0.80	85.63	< 0.0001**
X1X4	0.37	1.00	0.37	39.35	< 0.0001**
X2X3	0.07	1.00	0.07	7.19	0.02*
X2X4	0.06	1.00	0.06	6.78	0.02*
X3X4	1.96	1.00	1.96	210.94	< 0.0001**
X1 ²	11.46	1.00	11.46	1232.39	< 0.0001**
X2 ²	3.60	1.00	3.60	386.81	< 0.0001**
X3 ²	7.08	1.00	7.08	760.90	< 0.0001**
X4 ²	1.94	1.00	1.94	208.58	< 0.0001**
Residual	0.13	14.00	0.01		
Lack of Fit	0.12	10.00	0.01	4.74	0.07
Pure Error	0.01	4.00	0.00		
Cor Total	21.52	28.00			
R ²	0.9939				
C.V.%	1.3				

**extremely significant ($p < 0.01$); *significant ($p < 0.05$)

All four variables could affect the extraction yield of SVP ($p < 0.01$) (Table 2). Based on the p value, extraction time was the most significant variable, followed by enzyme–solid ratio, extraction temperature and water–solid ratio. Meanwhile, all interaction effects of any two variables, except for X1X2, were significant ($p < 0.05$). Furthermore, all quadratic effects were also extremely significant ($p < 0.01$).

Response surface analysis

The 3D response surface plots provided a graphical representation of the model (Fig. 1). When the variables increased, the yield of SVP initially increased, and then decreased. The interaction effects of ratio of enzyme to raw material and extraction temperature on the SVP yield as the other two factors were fixed as shown in Fig. 1c. Extraction yield increased with the ratio of enzyme to raw material, and then remained static. Furthermore, as the extraction temperature increased, the yield initially increased and subsequently decreased; this was attributed to reaching, and then exceeding, an optimal temperature for the enzyme reaction. The modest interaction effect of the enzyme–solid ratio and the water–solid ratio with the other two factors fixed on the extraction yield with a gentle 3D surface in the given range of variables is shown in Fig. 1e, whereas the interaction effect of enzyme–solid ratio and extraction time with the other two factors fixed, in which the two factors increased first and levelled off later as the test variables, is also shown (Fig. 1f).

Verification of the predictive model

Based on 3D plots, the feasibility of the model equation for predicting the highest yield could be tested as follows: extraction duration, 68 min; extraction temperature, 51°C; ratio of enzyme to raw material, 1.6%; ratio of liquid to raw material, 20 mL/g; followed by ultrasonic treatment (180 W, 60°C, 60 min). Under these optimized conditions, the maximum predicted extraction yield of SVP was 9.01%, which was very close to the experimental yield ($8.94 \pm 0.38\%$, $n = 3$). There was no significant difference between the predicted and real values, which demonstrated that this model was appropriate and adequate to optimize SVP extraction.

Purification of fractions

Three main peaks (SVP-1, SVP-2 and SVP-3) were obtained from crude SVP through DEAE-52 cellulose column chromatography (Fig. 2). All fractions were further purified by Sephadex G-100 column chromatography, and then collected, dried and stored in a desiccator for further use.

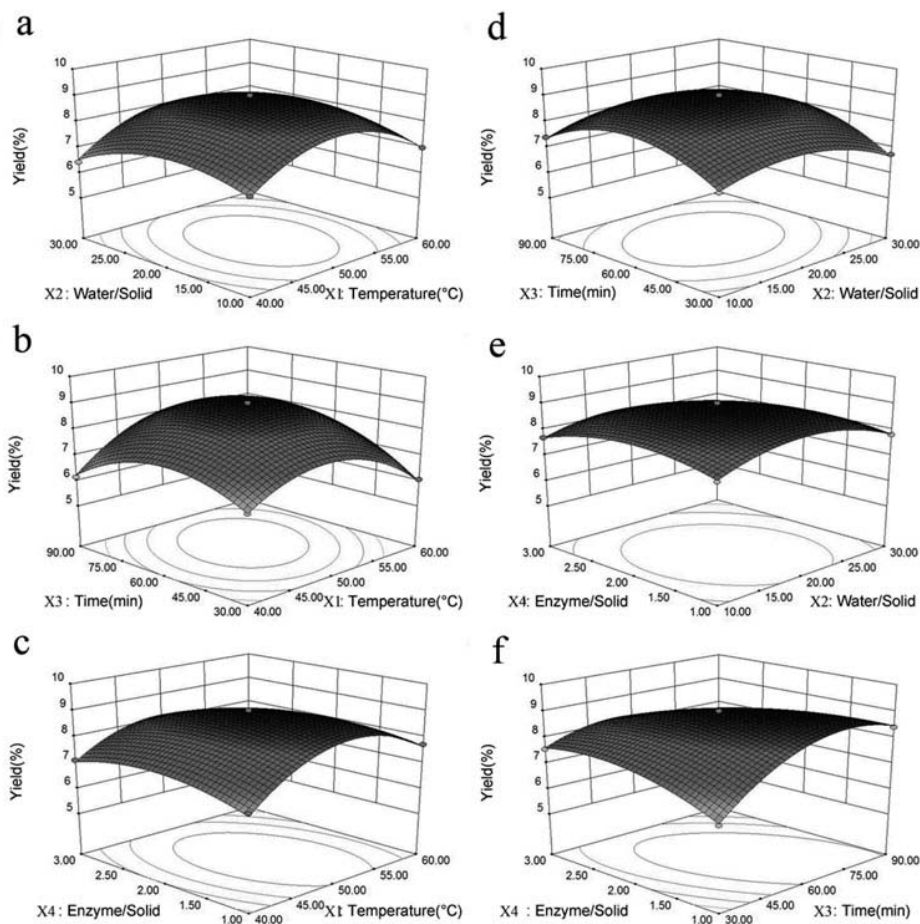


Figure 1. Response surface plots showing interaction effect of any two factors on total polysaccharide yields of *Setaria viridis*.

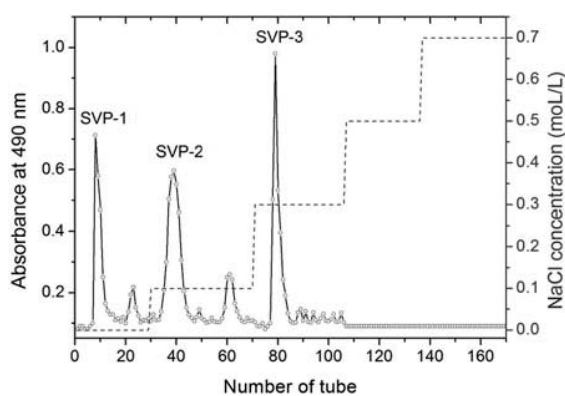


Figure 2. Anion-exchange chromatogram of crude SVP detected by phenol-sulphuric acid method at 490 nm. Column: DEAE52-cellulose (2.6×60 cm); flow rate: 0.6 mL/min; fraction volume: 5 mL. SVP-10, SVP-20 and SVP-30 were eluted by 0, 0.1 and 0.3 mol/L NaCl, respectively.

Preliminary characterization of polysaccharides

Chemical composition and molecular weight of SVP-1, SVP-2 and SVP-3 are shown in Table 3. The total sugar

contents of SVP-1, SVP-2 and SVP-3 were 95.67, 93.59 and 90.96%, respectively. The protein contents of SVPs were all below 1%. Based on the vitriol-carbazole reaction, the uronic acid contents of SVP-1, SVP-2 and SVP-3 were 0.15, 0.84 and 2.75%, respectively.

According to HPSEC analysis, SVP-1, SVP-2 and SVP-3 all displayed a single symmetrical peak, and their average molecular weights were 14.52, 89.21 and 28.14 kDa, respectively. These fractions had different monosaccharide composition and ratio. Both SVP-2 and SVP-3 consisted of glucose, galactose, mannose, xylose and rhamnose, but SVP-1 did not contain rhamnose. The major monosaccharides of SVP-3 were glucose and mannose, while SVP-1 and SVP-2 were glucose and xylose, respectively.

FTIR spectrum

The infrared spectrum of SVPs is shown in Fig. 3. There was a characteristic hydroxyl group at $3300\text{--}3500\text{ cm}^{-1}$ and a weak C-H band at approximately $2928\text{--}2990\text{ cm}^{-1}$.

Table 3. Chemical composition and molecular weight of SVP.

Sample	Total sugar (%)	Total protein (%)	Uronic acid (%)	Molecular weight (kDa)	Monosaccharide composition (%)				
					Glucose	Galactose	Mannose	Xylose	Rhamnose
SVP-1	95.67	0.14	0.15	14.52	35.93	12.10	9.22	39.14	nd
SVP-2	93.59	0.28	0.84	89.21	48.75	6.48	11.92	22.11	3.91
SVP-3	90.96	0.63	2.75	28.14	51.63	7.58	17.09	4.14	5.29

nd = not detected

The relatively strong absorption peaks around 1640 cm^{-1} and 1420 cm^{-1} were attributed to the presence of carboxylic groups, whereas the peak at $1000\text{--}1200\text{ cm}^{-1}$ was owing to C-O-C and C-O-H link bonds. Additionally, a characteristic band at 850 cm^{-1} was due to α -type glycosidic linkages.

Antioxidant activities of SVPs

DPPH and hydroxyl radical scavenging activity of SVPs

Free radicals played inevitable roles in regulating homeostasis and mediating stress responses *in vivo*. A small amount of radicals could initiate the defence system of the cell against infections while excessive free radicals might induce diabetes, cancer, neurodegenerative and cardiovascular diseases.^[27] Thus, exogenous antioxidants are highly important to keep the level of radicals within a limited range and to avoid oxidative damage.

The DPPH and hydroxyl radical scavenging activities of SVPs are shown in Fig. 4a and 4b. All samples showed a dose-dependent effect. At 2.4 mg/mL , SVP-3 had the highest DPPH radical and hydroxyl radical scavenging activity of 70.14 and 60.82%, followed by SVP-2 (51.60 and 49.93%) and SVP-1 (7.98 and

21.05%). It is reported that monosaccharides composition of polysaccharides was closely related to their antioxidant activity.^[28,29] In this study, SVP-3 with a high content of glucose and mannose exhibited strong radical scavenging activity.

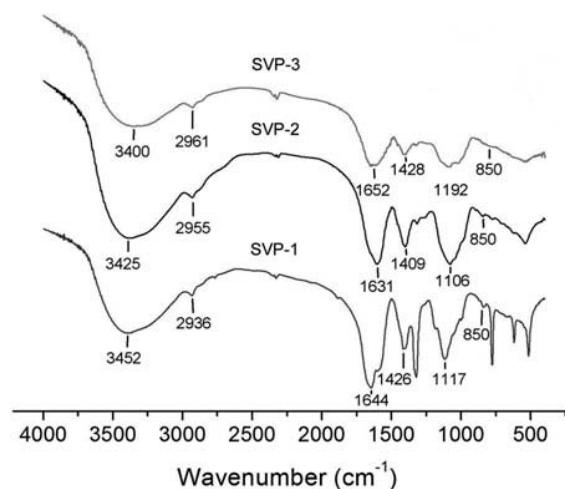
Reducing power of SVPs

Reducing power was generally related to the ability of donating hydrogen atoms.^[30] A higher absorbance at 700 nm indicated greater reducing power. The results are shown in Fig. 4c. The reducing power of all samples showed concentration-dependent effect. At 2.4 mg/mL , the reducing power of all samples was SVP-3 > SVP-2 > SVP-1. SVP-3 with medium molecular weight (28.14 kDa) had stronger antioxidant activity than SVP-2 and SVP-1 (89.21 and 14.52 kDa, respectively). Our results were in agreement with a previous study, which indicated medium molecular weight of tea polysaccharides possessed high antioxidant activity.^[31] Perhaps, this was due to the hydroxyl groups of polysaccharides, which could potentially provide hydrogen atoms and further eliminate free radicals.

Metal ions chelating capacity of SVPs

Transition metal ions can promote the generation of ROS, resulting in oxidative damage.^[32] To retard the oxidation reaction, chelation of transition metal ions by antioxidants is an alternative way. Iron chelating capacity of SVPs at different concentrations is shown in Fig. 4d. Iron chelating capacity of SVPs increased with concentration. At 2.4 mg/mL , SVP-2 showed the highest iron chelating capacity of 81.71%, followed by SVP-3 and SVP-1.

The polysaccharides with a moderate molecular weight might have more active sites to chelate metal ions. This result was consistent with the *Cyclina sinensis* polysaccharides.^[30,31] In fact, compounds with metal ion chelating activities usually were likely to contain the following functional groups: -OH, -SH, -COOH, $-\text{PO}_3\text{H}_2$, CO, $-\text{NR}_2$, -S- and -O-.^[33] Thus, polysaccharides with different molecular weights might have a wide range of active sites to chelate metal ions.

**Figure 3.** FTIR spectrometry of SVP-1, SVP-2 and SVP-3.

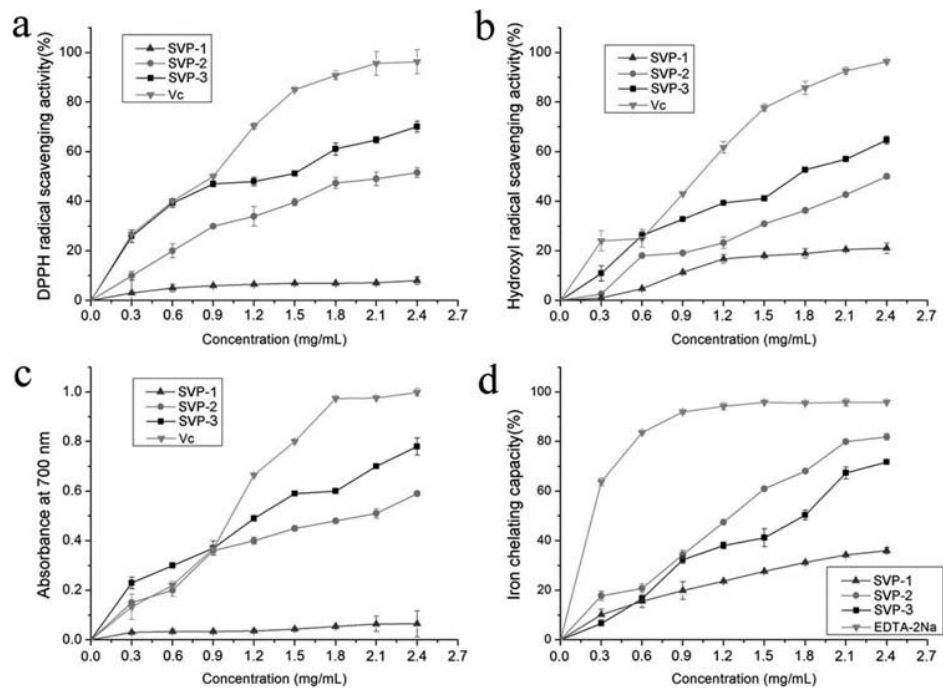


Figure 4. Effect of SVP-1, SVP-2 and SVP-3 on (a) DPPH radical, (b) hydroxyl radical, (c) reducing power, and (d) iron chelating capacity. Each value is mean \pm SD of triplicate measurements.

Conclusions

In the present study, UEAE of polysaccharides from *S. viridis* was optimized by BBD. The optimal extraction condition in the enzymatic hydrolysis process was performed as follows: extraction duration, 68 min; extraction temperature, 51°C; ratio of enzyme to raw material, 1.6%; ratio of liquid to raw material, 20 mL/g. Under these optimized conditions, the highest yield of crude SVP was $8.94 \pm 0.38\%$. Purified SVP fractions, SVP-1, SVP-2 and SVP-3, had similar FTIR spectra, but with distinct monosaccharide compositions, molecular weights and *in vitro* antioxidant activities. In addition, SVP-3 with the maximum uronic acid content and moderate molecular weight among those fractions had the strongest antioxidant activity. Further investigations regarding the *in vivo* antioxidant activities of SVP-3 are highly demanded.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was financially supported by the Double Support Programs of Sichuan Agricultural University.

References

- [1] Yuan, M.; Jia, X.J.; Ding, C.B.; Yuan, S.; Zhang, Z.W.; Chen, Y.E. (2014) Comparative studies on bioactive constituents in hawk tea infusions with different maturity degree and their antioxidant activities. *The Scientific World Journal*, 2014: 1–7.
- [2] Halliwell, B.; Gutteridge, J.M. (1986) Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Archives of Biochemistry and Biophysics*, 246 (2): 501–514.
- [3] Halliwell, B.; Gutteridge, J.M. (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, 219 (1): 1–14.
- [4] Ramarathnam, N.; Osawa, T.; Ochi, H.; Kawakishi, S. (1995) The contribution of plant food antioxidants to human health. *Trends in Food Science & Technology*, 6 (3): 75–82.
- [5] Hayashi, K.; Hayashi, T.; Kojima, I. (1996) A natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. *AIDS Research and Human Retroviruses*, 12 (15): 1463–1471.
- [6] Zong, A.Z.; Cao, H.Z.; Wang, F.S. (2012) Anticancer polysaccharides from natural resources: A review of recent research. *Carbohydrate Polymers*, 90 (4): 1395–1410.
- [7] Moradali, M.F.; Mostafavi, H.; Ghods, S.; Hedjaroude, G.A. (2007) Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). *International Immunopharmacology*, 7 (6): 701–724.

- [8] Mateos-Aparicio, I.; Mateos-Peinado, C.; Jiménez-Escrig, A.; Rupérez, P. (2010) Multifunctional antioxidant activity of polysaccharide fractions from the soybean byproduct okara. *Carbohydrate Polymers*, 82 (2): 245–250.
- [9] Yan, Y.L.; Yu, C.H.; Chen, J.; Li, X.X.; Wang, W.; Li, S.Q. (2011) Ultrasonic-assisted extraction optimized by response surface methodology, chemical composition and antioxidant activity of polysaccharides from *Tremella Mesenterica*. *Carbohydrate Polymers*, 83 (1): 217–224.
- [10] Liao, N.B.; Zhong, J.J.; Ye, X.Q.; Lu, S.; Wang, W.J.; Zhang, R.H.; Xu, J.; Chen, S.G.; Liu, D.H. (2015) Ultrasonic-assisted enzymatic extraction of polysaccharide from *Corbicula fluminea*: Characterization and antioxidant activity. *LWT-Food Science and Technology*, 60 (2): 1113–1121.
- [11] Maran, J.P.; Priya, B. (2015) Ultrasound-assisted extraction of pectin from sisal waste. *Carbohydrate Polymers*, 115: 732–738.
- [12] Fan, L.; Ma, J.; Chen, Y.H.; Chen, X.Q. (2014) Antioxidant and antimicrobial phenolic compounds from *Setaria viridis*. *Chemistry of Natural Compounds*, 50 (3): 433–437.
- [13] Gong, J.; Ni, S.F.; Li, W.J.; Luo, R.F.; Hou, E.T.; Zhu, E. L.; Zhao, B. (2010) Overview of the pharmaceutical research on *Setaria Beavv*. *Journal of Anhui Agricultural Sciences*, 38 (15): 66–68.
- [14] Luo, S.Q.; Nie, Y.; Liu, L.L.; Yang, Q.H.; Zhang, C.R.; Jiang, H.; Chen, Y.F. (2007) Experimental study on traditional mongolian material medica of *Setaria viridis* (L.) Beauv. *Acta Academiae Medicinae Neimongol*, 29 (3): 190–191.
- [15] Wu, G.; Yi, Y.; Mao, Z. (2008) Clinical observation of green bristle grass curing verruca vulgaris and clavus. *Chinese Journal of Ethnomedicine and Ethnopharmacy*, 4: 48–49.
- [16] Zhang, A.W.; Luo, S.Q.; Liu, L.L. (2010) Extraction, identification and determination of total sugar from *Setaria viridis* (L.) Beauv. fruits. *Acta Academiae Medicinae Neimongol*, 32 (5): 472–474.
- [17] Xian, J.C.; Gao, W. (2012) Study on extraction of total polyphenol in *Setaria viridis* (L.) Beauv. and its anti-oxidation activity. *Journal of Sichuan Agricultural University*, 30 (3): 348–351.
- [18] Zhang, A.W.; Liu, L.L.; Luo, S.Q.; Wang, L.B.; Zhao, Q. S. (2013) Study on technology for extracting total tannin from *Setaria viridis* (L.) Beauv. ripe fruits by orthogonal test. *Acta Academiae Medicinae Neimongol*, 35 (3): 216–220.
- [19] Cheng, H.R.; Feng, S.L.; Shen, S.A.; Zhang, L.; Yang, R. W.; Zhou, Y.H.; Ding, C.B. (2013) Extraction, antioxidant and antimicrobial activities of *Epimedium acuminatum* Franch. polysaccharide. *Carbohydrate Polymers*, 96 (1): 101–108.
- [20] Jia, X.J.; Ding, C.B.; Yuan, S.; Zhang, Z.W.; Du, L.; Yuan, M. (2014) Extraction, purification and characterization of polysaccharides from Hawk tea. *Carbohydrate Polymers*, 99: 319–324.
- [21] Zeng, W.C.; Zhang, Z.; Jia, L.R. (2014) Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*). *Carbohydrate Polymers*, 108: 58–64.
- [22] Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, 193 (1): 265–275.
- [23] Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.; Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28 (3): 350–356.
- [24] Lin, Y.; Huang, L.J.; Tian, G.Y. (1999) An improved method for the quantitative determination of uronic acid. *Chinese Herbal Medicine*, 30 (11): 817–819.
- [25] Jia, X.J.; Dong, L.H.; Yang, Y.; Yuan, S.; Zhang, Z.W.; Yuan, M. (2013) Preliminary structural characterization and antioxidant activities of polysaccharides extracted from Hawk tea (*Litsea coreana* var. lanuginosa). *Carbohydrate Polymers*, 95 (1): 195–199.
- [26] Wang, J.; Zhang, Q.; Zhang, Z.; Li, Z. (2008) Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. *International Journal of Biological Macromolecules*, 42 (2): 127–132.
- [27] Fiaschi, T.; Chiarugi, P. (2012) Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. *International Journal of Cell Biology*, 2012: 1–8.
- [28] Chen, H.X.; Zhang, M.; Qu, Z.S.; Xie, B.J. (2008) Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia Sinensis*). *Food Chemistry*, 106 (2): 559–563.
- [29] Yang, L.; Zhang, L.M. (2009) Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers*, 76 (3): 349–361.
- [30] Jiang, C.X.; Xiong, Q.P.; Gan, D.; Jiao, Y.P.; Liu, J.; Ma, L.P.; Zeng, X.X. (2013) Antioxidant activity and potential hepatoprotective effect of polysaccharides from *Cyclina sinensis*. *Carbohydrate Polymers*, 91 (1): 262–268.
- [31] Wang, Y.F.; Yang, Z.W.; Wei, X.L. (2012) Antioxidant activities potential of tea polysaccharide fractions obtained by ultra filtration. *International Journal of Biological Macromolecules*, 50 (3): 558–564.
- [32] Saiga, A.; Tanabe, S.; Nishimura, T. (2003) Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, 51 (12): 3661–3667.
- [33] Yuan, Y.V.; Bone, D.E.; Carrington, M.F. (2005) Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated in vitro. *Food Chemistry*, 91 (3): 485–494.