International Food Research Journal 31(5): 1134 - 1143 (October 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Novel alkali extraction, optimisation, characterisation, and antioxidant activity of polysaccharides from Foshou yam

[1]Hu, T., [1]Wang, F., [1]Liu, B., [1]Zheng, Y.,
[1]*Wu, P. and [1,2]*Li, S.

[1]Hubei Provincial Key Laboratory of Economic Forest Germplasm Improvement and Comprehensive Utilization of Resources, College of Biology and Agricultural Resources, Huanggang Normal University, Huanggang, 438000, China
[2]Department of Food Science, Rutgers University, New Brunswick, New Jersey, USA

Article history

Received:
29 January 2024
Received in revised form:
12 July 2024
Accepted:
12 August 2024

Keywords

Foshou yam,
Dioscorea opposita Thunb.,
polysaccharide,
alkali extraction,
antioxidant activity,
physicochemical characterisation

Abstract

Foshou yam (*Dioscorea opposita* Thunb.), cultivated as a vegetable in the region of eastern Hubei Province, China, is a nutritious delicacy. Polysaccharides in the genus *Dioscorea* play important roles in health-promoting activities. Previous extraction methods used to obtain polysaccharides from Foshou yam (FYP) included water and enzymes, but not alkali conditions. The extraction methods greatly influenced the structure and biological properties of polysaccharides. Hence, in the present work, the alkali extraction of FYP was explored and optimised using the response surface method. The characterisation and antioxidant activity of FYP were also investigated. The results showed that the optimal extraction conditions were as follows: sodium hydroxide concentration, 1.0 mol/L; extraction time, 8 h; and solvent-to-material ratio, 10 mL/g. Under these conditions, the FYP extraction yield was 64.52 ± 0.23%. FYP contained 91.70% of the total sugar with a minimal amount of protein. FT-IR analysis revealed the presence of α-D-glucopyranose in FYP. Congo Red tests indicated that the triple helical conformation in FYP did not exist. In addition, *in vitro* antioxidant activity experiments revealed that FYP exhibited good scavenging capacity on DPPH and superoxide anion free radicals. These results indicated that the FYP can be used as a novel natural antioxidant in the functional food industries.

DOI
https://doi.org/10.47836/ifrj.31.5.05

Introduction

There are more than 600 species of yam (*Dioscorea* spp.) in the family Dioscoreaceae, including *D. opposita* Thunb. or *D. oppositifolia* L. (Chinese or Japanese yam), *D. nipponica* Thunb. (Japanese yam), *D. polystachya* Turczaninow (white or Chinese yam), *D. alata* (purple, greater, or water yam), *D. cayenensis* Lam.-Holl (yellow yam or African yam), and many other yams that are regionally grown. As an ancient edible and medicinal tuber crop, the yam family was first described in the old book "Classic of Material Medica" (Shen-nung Pen-tsao Ching), in which several pharmacological effects, such as asthma relief and diarrhoea effects, were documented (Huang *et al*., 2016). Yam contains many bioactive components, including bioactive polysaccharides, amino acids and proteins, polyphenols, flavonoids, saponins, terpenoids, and allantoin, among others (Salehi *et al.*, 2019). Polysaccharides from the family Dioscoreaceae are one of the most characteristic phytochemicals because of their diverse biological activities. For instance, mucilage polysaccharides extracted from *D. opposita* exhibited hypoglycaemic, immunoregulatory, antitumor, and antioxidative activities (Ju *et al.*, 2014; Hao, 2016a).

There are several methods for extracting polysaccharides from yam. Commonly employed methods include high temperature accelerated method which assists in the dissolution of polysaccharides from cell walls, ultrasonic assisted water extraction which facilitates the release of intracellular polysaccharides, enzymatic assisted water extraction which destroys plant cell walls, and increases the solubility of polysaccharides, and alkali

*Corresponding author.
Email: 115479165@qq.com ; shiming@rutgers.edu

treatment which breaks cell walls, and dissolves the intracellular polysaccharide. The mechanism usually involves breaking the cell walls and stimulating the release of yam polysaccharides.

Foshou yam (*D. opposita* Thunb.) is considered a rare species of the yam family, and known for its high nutritional value and rich dietary fibre. It is also referred to as the 'Ginseng of the Dabie Mountains' or 'Food of the Gods' in the central part of China. Foshou yam is regarded as an ideal delicacy for health and longevity because it contains various nutrients, such as polysaccharides, proteins, starches, polyphenols, 19 different types of amino acids, diosgenin, allantoin, vitamins, and trace elements. Among them, polysaccharides have become a topic because of their high biological activities. It has been reported that yam polysaccharides possess a variety of biological activities, such as immunomodulatory (Hao, 2016b; Li *et al.*, 2017; Huang *et al.*, 2020a), hypoglycaemic (Cheng *et al.*, 2019), antiaging (Wang *et al.*, 2020), antioxidant (Zhou, 2021; Liu *et al.*, 2022), antitumor (Huang *et al.*, 2020b), and regulatory effects on gut microbiota diversity (Li *et al.*, 2020).

To date, many studies have focused on finding the optimal method for extracting polysaccharides to further study their physicochemical characteristics and biological activities. The most commonly used methods for the extraction of yam polysaccharides include hot water extraction, enzyme extraction, ultrasound- and microwave-assisted extraction (Liu *et al.*, 2019; 2022; Huang *et al.*, 2020b). However, hot water extraction has the disadvantages of longer time, higher temperature, and lower extraction yield. It has been reported that the optimal hot water extraction conditions are an extraction temperature of 80°C, an extraction time of 3 h, and a water-to-material ratio of 10:1 (w:w), and the extraction yield of yam polysaccharides is approximately 10% under these conditions (Huang *et al.*, 2020a). The enzymes used in the extraction process can improve the extraction yield, and shorten the extraction time, but they are easily affected by many factors, such as extraction temperature, extraction time, pH value, and other factors. Ultrasound-assisted extraction involves the crushing of plant cell walls by ultrasonic wave, so that plant polysaccharides in cells can dissolve. Although the extraction yield can be greatly improved by reducing the reaction time, the problem of solvent recovery must be addressed. Microwave extraction has a high penetrating power, high selectivity and heating efficiency, and a high extraction yield, but the risk of microwave leakage is a challenge. It has been reported that a dilute alkali solution used to extract polysaccharides, such as lentinan and polysaccharide from *Lignosus rhinocerotis* sclerotia at low temperatures, leads to a high extraction yield with a high degree of purity (Zhang *et al.*, 2011; Hu *et al.*, 2017a; Sun *et al.*, 2022).

Currently, the main methods employed for Foshou yam polysaccharide (FYP) extraction include water and enzyme extraction. However, studies related to the alkali extraction method have not been reported to date. Therefore, the present work aimed to determine the optimal alkali extraction conditions for FYP using response surface methodology (RSM) to obtain the highest yield. The extraction of polysaccharides *via* different methods affects their characterisation and biological activities (Hu *et al.*, 2021b; 2022; Tang *et al.*, 2023). Hence, FYP was characterised by chemical content analysis, UV-vis, Fourier transform infrared spectrometer (FT-IR), and Congo Red tests. Moreover, the antioxidant activity of FYP was investigated by determining its DPPH and superoxide anion free radical scavenging activities. The obtained results would provide a theoretical basis for the utilisation of FYP as an antioxidant.

Materials and methods

Materials and reagents

Foshou yam was procured from the Hubei Province of Wuxue City (Wuxue, China). Dialysis bags were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Sodium hydroxide, sodium bicarbonate, sulphuric acid, anhydrous ethanol, Folin phenol reagent, potassium bromide, and glucose were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1,1-Diphenyl-2-trinitrobenzene hydrazine (DPPH) was purchased from Tishiai Huacheng Industrial Development Co., Ltd. (Shanghai, China). Tris-HCl was purchased from Shanghai Qiaoxing Trading Co., Ltd. (Shanghai, China). Pyrogallol was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Congo Red was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). All chemical reagents were of analytical grade.

Preparation of Foshou yam powder (FYP)

Fresh Foshou yams were washed, peeled, sliced into 2 - 3 mm slices, and then soaked in 0.3% Na_2SO_3 solution for 3 h to retain the colour. After washing, the Foshou yam slices were heated at 45°C in an oven until only 10% moisture remained. The dry Foshou yam slices were powdered using a grinder, and sieved through 80 mesh. Finally, the Foshou yam powder (FYP) was transferred to a sealed bag, and stored in a drying vessel.

Extraction of FYP

A certain volume and concentration of NaOH solution were added to the FYP, and kept at 4°C for a certain period of time. After centrifugation (3,500 rpm, 10 min), the supernatant was collected and neutralised by adding 4 M or 8 M hydrochloric acid, and then dialysed in a dialysis bag (M_w: 8,000 – 12,000 Da) for 7 d against distilled water to remove inorganic salts. Finally, the solution was lyophilised to obtain FYP. The extraction yield of FYP was determined using Eq. 1:

$$\text{Extraction yield} = \frac{\text{Mass of FYP (g)}}{\text{Mass of Foshou yam power (g)}} \times 100\% \quad \text{(Eq. 1)}$$

Single factor test

In the single factor test, the NaOH concentration, extraction time, and solvent-to-material ratio were selected for the optimisation of FYP extraction. When the concentration of NaOH ranged from 0.1 to 1.3 mol/L, the extraction time and solvent-to-material ratio were 8 h and 10 mL/g, respectively. When the extraction time was changed from 4 to 12 h, the concentration of NaOH and the solvent-to-material ratio were 0.9 mol/L and 10 mL/g, respectively. When the solvent-to-material ratio changed from 6 to 16 mL/g, the NaOH concentration and extraction time were 0.9 mol/L and 8 h, respectively.

Response surface optimisation experiment

Response surface methodology (RSM) was used to investigate the effect of the concentration of NaOH (A), extraction time (B), and solvent-to-material ratio (C) on the extraction yield of FYP (Y). The coding values and levels of the three factors of Box and Behnken design (BBD) are shown in Table 1. Twenty combinations were produced by BBD as shown in Table 2.

Table 1. Factors and levels of Box-Behnken design.

Level	Factor		
	Concentration of NaOH (mol/L) (A)	Extraction time (h) (B)	Solvent-to-material ratio (mL/g) (C)
-1	0.7	6	8
0	0.9	8	10
1	1.1	10	12

Table 2. Box-Behnken design and results.

Test no.	A (mol/L)	B (h)	C (mL/g)	Response value (%)
1	1.10	10.00	8.00	56.03
2	0.90	8.00	10.00	61.23
3	0.90	8.00	10.00	65.04
4	0.70	10.00	8.00	48.60
5	0.70	6.00	12.00	53.87
6	0.90	8.00	10.00	62.65
7	0.90	8.00	10.00	59.63
8	0.90	8.00	10.00	64.45
9	0.90	8.00	10.00	46.67
10	0.70	6.00	8.00	51.21
11	0.70	10.00	12.00	52.87
12	1.10	6.00	12.00	56.59
13	0.90	8.00	10.00	64.73
14	0.90	8.00	10.00	63.87
15	1.10	10.00	12.00	57.86
16	0.90	10.00	10.00	49.33
17	0.90	8.00	10.00	64.10
18	0.90	8.00	10.00	54.59
19	1.10	6.00	8.00	53.32
20	0.90	8.00	8.00	54.96

Verification experiment

The actual extraction yield under the optimised extraction conditions of FYP was determined, and the process was repeated three times for reliability analysis to verify the accuracy of the predicted extraction yield of FYP.

Chemical composition analysis

The total sugar content of FYP was measured using the phenol-sulphuric acid method with glucose as a standard (Hu *et al.*, 2017b). Briefly, anhydrous glucose (10 mg) was weighed to prepare a 0.1 mg/mL glucose standard solution. Next, 0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL of the standard glucose solutions were placed in the test tubes, and distilled water added to 2.0 mL, respectively. Then, 1.0 mL of 5%

phenol and 5.0 mL of concentrated sulphuric acid were added. The mixture was shaken and kept at room temperature for 10 min, and the absorbance value was measured at 490 nm using a visible spectrophotometer. The standard curve was drawn with the absorbance value as the ordinate, and the glucose concentration as the abscissa. FYP (10 mg) was dissolved in 10 mL distilled water to prepare a 1.0 mg/mL sample solution. Then, 1.0 mL sample solution was removed, and 1.0 distilled water was added. The remaining steps were the same as earlier described, and the total sugar content of FYP was calculated against the standard curve.

The protein content of FYP was determined by the modified Lowry method using bovine serum albumin as the standard (Hu *et al.,* 2017b). Briefly, bovine serum protein (10 mg) was weighed to prepare a 1 mg/mL bovine serum protein standard solution. Then, 0, 0.01, 0.03, 0.05, 0.07, and 0.09 mL of the standard solution were added to 0.1 mL of distilled water, respectively. Next, 1 mL of Folin A solution was added, and the solution was mixed well. After being kept in a 37°C water bath for 15 min, 0.1 mL of Folin B solution was added. The mixture was shaken and kept at 37°C water bath for 30 min, and the absorbance value was measured at 750 nm using a visible spectrophotometer. The standard curve was drawn with the absorbance value as the ordinate, and the protein concentration as the abscissa. Next, 1.0 mL FYP solution in the test tube was taken, and the remaining steps were the same as earlier described. The protein content of FYP was determined against the standard curve.

UV-vis analysis

The UV-vis spectrum of FYP at a concentration of 1.0 mg/mL was scanned using a UV-vis spectrophotometer (UV-2600, Shimadzu, Japan) from 200 - 600 nm (Liu *et al.,* 2022).

FT-IR analysis

The FT-IR spectrum of FYP was recorded by an FT-IR spectrometer (Nicolet 6700, Nicolet, UK) using the KBr-pellet method at the frequency range of 400 to 4000 cm^{-1} (Ju *et al.,* 2014).

Congo Red test

Following the method of Hu *et al*. (2021a), FYP was dissolved in 2 mL of NaOH solution in the concentration range of 0 - 0.6 mol/L. Then, the Congo Red was added at a final concentration of 91 μM, and the mixture was kept at room temperature for approximately 20 min. The maximal absorption wavelength of these mixtures was recorded using a UV-vis spectrophotometer (UV-2600, Shimadzu, Japan) from 400 - 600 nm.

In vitro antioxidant activity of FYP

The *in vitro* antioxidant activity of FYP was evaluated based on the scavenging capacity of DPPH and superoxide anion free radical.

Scavenging capacity of DPPH free radical

The DPPH free radical scavenging effect of FYP was determined following our previous report with some modifications (Wu *et al.,* 2020). Different concentrations of FYP solution (20, 40, 60, 80, and 100 μg/mL) were first prepared. Then, 4.0 mL of 0.04 mg/mL DPPH ethanol solution was added to 2.0 mL of the previously prepared FYP solution with different concentrations in the test tubes. The mixture was mixed well, reacted in the dark for 30 min at room temperature, and then the absorbance value was measured at 517 nm. This experiment was repeated three times. The DPPH free radical scavenging rate of FYP was calculated using Eq. 2:

$$\text{DPPH scavenging rate (\%)} = \frac{A_1-(A_2-A_3)}{A_1} \times 100\% \quad \text{(Eq. 2)}$$

where, A_1 = absorbance value of the blank control determined by replacing the sample solution with anhydrous ethanol of the same volume; A_2 = absorbance value of the sample solution; and A_3 = absorbance value of the background determined by replacing the DPPH solution with anhydrous ethanol of the same volume.

Scavenging capacity of superoxide anion free radicals

The superoxide anion free radical scavenging effect of FYP was measured following our previous report with some modifications (Wu *et al.,* 2020). Firstly, 4.5 mL of 50 mmol/L Tris-HCl (pH = 8.2) was added to 2.0 mL FYP solution with different concentrations in the test tubes, and incubated at 25°C water bath for 10 min. Then, 0.5 mL of 25 mmol/L pyrogallol solution was added to the solution. After mixing, the reaction was terminated by adding 0.5 mL of 8.0 mol/L HCl. The absorbance value was measured at 320 nm, and distilled water and V_C were used as the blank control and positive control,

respectively. The scavenging rate of FYP on DPPH free radical was calculated using Eq. 3:

$$\text{Superoxide anion free radical scavenging rate (\%)} = \frac{A_1-(A_2-A_3)}{A_1} \times 100 \quad \text{(Eq. 3)}$$

where, A_1 = absorbance value of the blank control determined by replacing the sample solution with distilled water of the same volume; A_2 = absorbance value of the sample solution; and A_3 = absorbance value of the background determined by replacing the pyrogallol solution with distilled water of the same volume.

Statistical analysis

All the data are expressed as the mean ± standard deviation (n = 3). Origin 2017 was used for graphs plotting, and SPSS 26.0 was used for data processing and significance analysis.

Results and discussion

Extraction of FYP under alkali conditions

To date, the main extraction methods for FYP are hot water and enzymatic extraction. Alkali extraction has not been used for the extraction of FYP. Hence, sodium hydroxide (NaOH) solution was used as a solvent to extract FYP, and the influence of three factors, namely, the NaOH concentration, extraction time, and solvent-to-material ratio on the extraction yield was investigated in the present work. The optimal alkali extraction conditions of FYP were determined by RSM. Moreover, the characterisation and antioxidant activity of FYP were studied.

Optimisation of FYP extraction conditions

Single factors

The effects of NaOH concentration, extraction time, and solvent-to-material ratio on the yield of FYP are shown in Figure 1. As illustrated in Figure 1A, the extraction yield of FYP increased from 33.06 to 65.12% as the concentration of NaOH increased from 0.1 to 0.9 mol/L, reaching a maximum when the concentration of NaOH was 0.9 mol/L, and then decreased with a further increase in the concentration of NaOH. The reason may be that the alkali would break the cell walls, leading to the polysaccharide transfer into the solvent. If the concentration of NaOH was too high, the structure of FYP would be easily destroyed, thereby resulting in a reduced extraction yield. Hence, a suitable concentration of NaOH was determined to be 0.9 mol/L. The extraction yield of FYP increased to a peak at 8 h, and then decreased (Figure 1B). The reason was that an extended extraction period caused the degradation of FYP, leading to a decrease in the extraction yield. Therefore, an optimal extraction time of 8 h was selected. As shown in Figure 1C, the extraction yield of FYP increased when the solvent-to-material ratio ranged from 6 to 10 mL/g, and reached a maximum at 10 mL/g. A further increase in the solvent-to-material ratio caused a decrease in the extraction yield of FYP. When the ratio of solvent-to-material was too high, some other substances started to dissolve, thus hindering the precipitation of polysaccharides and a consequent decrease in the extraction yield of FYP. Therefore, a suitable solvent-to-material ratio of 10 mL/g was used.

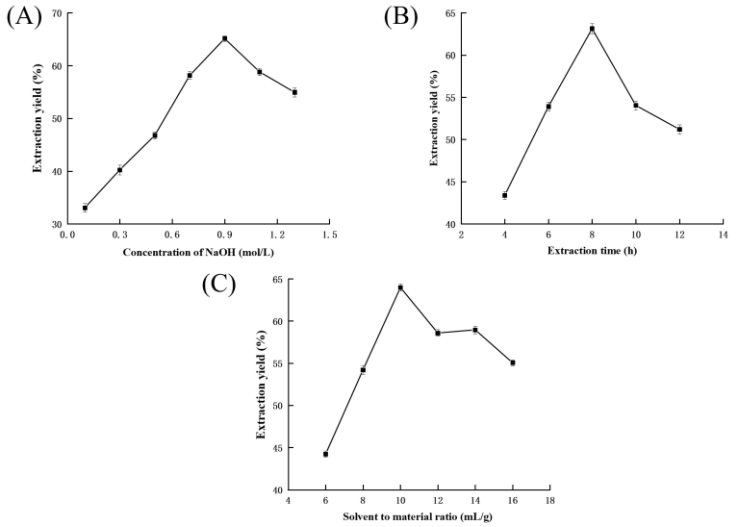

Figure 1. Effect of NaOH concentration **(A)**, extraction time **(B)**, and solvent-to-material ratio **(C)** on FYP extraction yield.

ANOVA of RSM

Based on the single-factor experimental results, three factors, *i.e.*, NaOH concentration (A), extraction time (B), and solvent-to-material ratio (C) were selected to optimise the FYP extraction process by RSM. The levels of the experimental factors are shown in Table 1. Based on the multiple regression analysis of the experimental data in Table 2, the extraction yield (Y) can be predicted using Eq. 4:

$$Y = 64.14 + 1.88A + 0.35B + 1.65C + 0.95AB - 0.23AC + 0.022BC - 2.49A^2 - 5.71B^2 - 2.14C^2 \quad \text{(Eq. 4)}$$

where, Y = extraction yield of FYP, A = NaOH concentration, B = extraction time, and C = solvent-to-material ratio.

The analysis of variance results are displayed in Table 3. The significance of the regression model can be measured by the values of *p* and F. The model was highly significant as the F-value was 85.43 and $p < 0.0001$. The lack of fit *p*-value (0.3699) was > 0.05, which indicated that the lack of fit was insignificant. The determination coefficient (R^2 = 0.9872) and adjusted determination coefficient (R^2_{adj} = 0.9756) were close to one, implying that the model was reliable, and could be applied to predict the optimum extraction conditions for FYP. The independent variables (A and C) and quadratic terms (A^2, B^2, and C^2) were highly significant ($p < 0.0001$), and the interaction term (AB) had a significant effect ($p < 0.05$) on the extraction yield of FYP. The other terms (B, AC, and BC) had no significant ($p > 0.05$). In addition, the order of the three factors affecting the extraction yield of FYP was A > C > B.

Table 3. Box-Behnken design variance analysis.

Source	Sum of squares	df	Mean square	F-value	*p*-value	Significance
Model	647.71	9	71.97	85.43	<0.0001	**
A	48.46	1	48.46	57.52	<0.0001	**
B	1.72	1	1.72	2.04	0.1838	
C	37.34	1	37.34	44.32	<0.0001	**
AB	7.21	1	7.21	8.55	0.0152	*
AC	0.42	1	0.42	0.50	0.4953	
BC	0.003	1	0.003	0.003	0.9470	
A2	89.32	1	89.32	106.03	<0.0001	**
B2	469.97	1	469.97	557.88	<0.0001	**
C2	66.08	1	66.08	78.44	<0.0001	**
Residual	8.42	10	0.84			
Lack of Fit	4.87	5	0.97	1.37	0.3699	
Pure Error	3.56	5	0.71			
Total Regression	656.13	19				

*significant difference at $p < 0.05$; **highly significant difference at $p < 0.0001$.

Response surface plot analysis

The three-dimensional (3D) response surface reflecting the effects and their interaction on the yield of FYP is displayed in Figure 2. Figure 2A shows the effect of the NaOH concentration (A), extraction time (B), and their interaction on the yield of FYP. The FYP yield increased with increasing NaOH concentration (A), and reached a maximum value when the NaOH concentration reached a certain value, after which the FYP yield tended to decrease. When the concentration of NaOH was set, the FYP yield also increased with increasing extraction time (B), reached a peak at a certain value of extraction time (B), and decreased when the extraction time exceeded a certain value. Too high concentration of NaOH and too long extraction time can lead to the degradation of FYP, resulting in a decrease in the FYP yield. Likewise, a similar trend was found in Figures 2B and 2C. In addition, the steepness of the response surface was proportional to the significance of the interaction; the steeper the surface of the 3D plot, the greater the impact of this factor on the yield of FYP. Figure 2A shows that when the extraction time (B) was fixed, the change in FYP yield with the solvent-to-material ratio (C) was relatively flat, suggesting that the influence of C was smaller than that of A. Similarly, Figure 2B shows the influence of B was smaller than that of A, and the influence of B

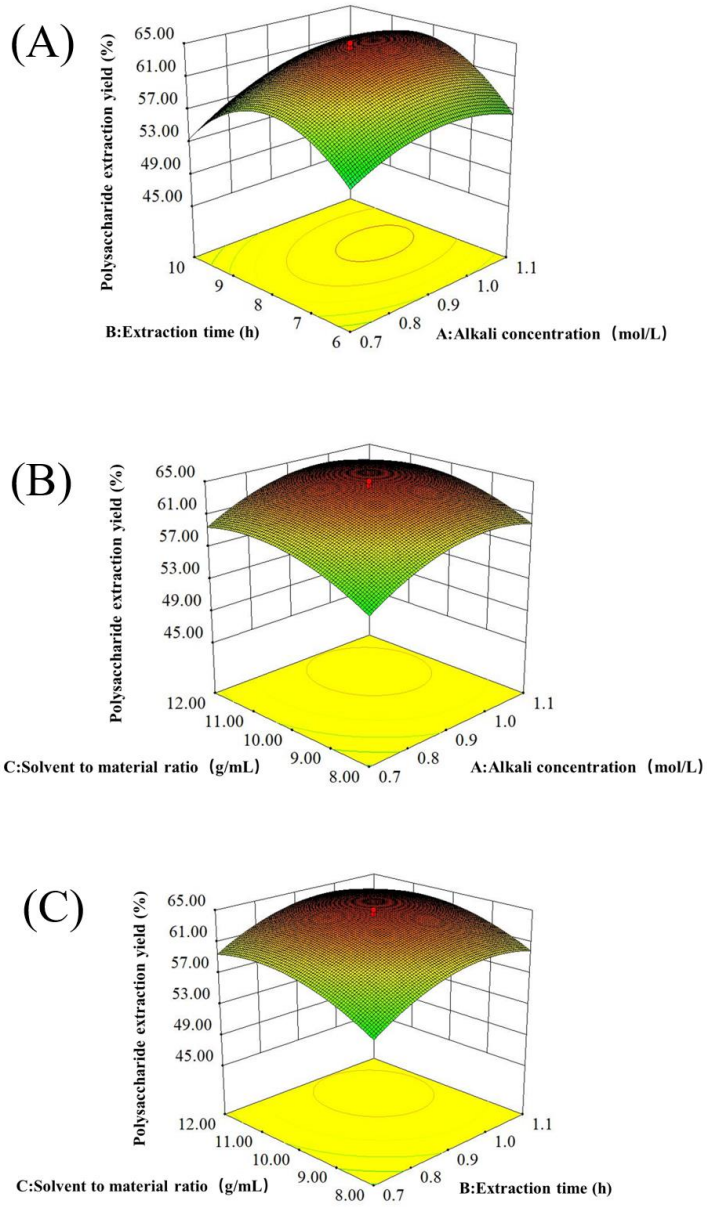

Figure 2. Response surface plots showing the effect of alkali concentration **(A)**, extraction time **(B)**, and solvent-to-material ratio **(C)** on FYP extraction yield.

was smaller than that of C as shown in Figure 2C. In summary, the order of influence of the three factors on the extraction yield of FYP was as follows: A > C > B, which was consistent with the results of ANOVA.

Therefore, the optimum extraction conditions for the maximum extraction yield of FYP were a NaOH concentration of 0.97 mol/L, an extraction time of 8.13 h, and a solvent-to-material ratio of 10.73 mL/g. Under these conditions, the extraction yield of FYP was predicted to be 64.81%. Considering the practical operation, the conditions were adjusted in the actual experiment as follows: the concentration of NaOH was 1.0 mol/L, the extraction time was 8 h, and the solvent-to-material ratio was 10 mL/g. The average extraction yield of FYP was 64.52 ± 0.23% in three parallel experiments conducted under the above conditions, which was close to the predicted value, indicating that the model could be used for the prediction of the extraction yield of FYP.

Characterisation of FYP

Analysis of chemical composition

The regression equation of the glucose standard curve was Y = 0.3341X-0.0354 (R^2 = 0.9919), and the total sugar content of FYP was calculated to be 91.70 ± 0.23%. The regression equation of the protein standard curve was Y = 0.3115X+0.2325 (R^2 = 0.9951), and the absorbance of the 1 mg/mL FYP solution was 0.071 ± 0.035 at 750 nm, which indicated that FYP had a minimal amount of protein. In addition, as shown in Figure 3, there was a weak absorption at 260 or 280 nm in the UV-vis spectrum of FYP, indicating that FYP contained a small amount of protein, which was consistent with the results of protein content determination (Zhou, 2021).

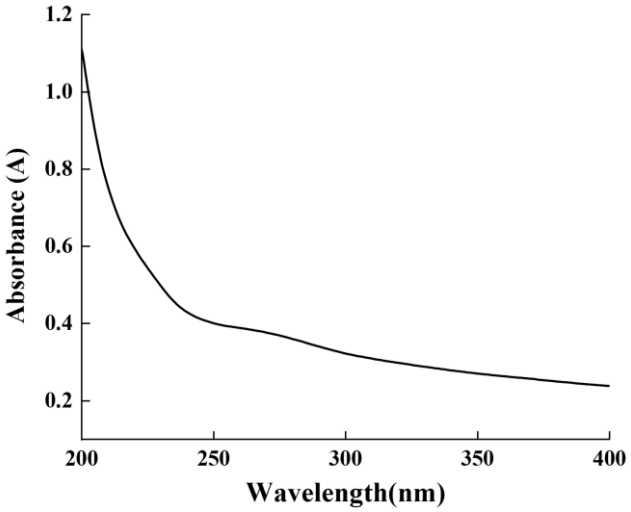

Figure 3. UV-vis spectrum of FYP.

FT-IR analysis

FT-IR can provide useful information about the characteristic functional groups of polysaccharides, and has typically been used to analyse their structures. As shown in Figure 4, the spectrum of FYP showed a strong absorption peak at approximately 3386 cm^{-1}, which corresponded to the O-H stretching vibration of the polysaccharide hydroxyl group, whereas a weak absorption peak at approximately 2928 cm^{-1} corresponded to the C-H stretching vibration, including -CH, -CH_2, and CH_3 (Yang *et al.*, 2015;

Feng *et al.*, 2022). The absorption peak at around 1648 cm^{-1} corresponded to the C=O stretching vibration of the sugar ring, and the absorption peak at 1373 cm^{-1} corresponded to the C-H variable angle vibration (Liu *et al.*, 2022). The absorption peaks at 1000 - 1200 cm^{-1} corresponded to the C-O-C stretching vibrations of glycosidic linkages and C-O-H stretching vibrations of side groups in FYP. The absorption peaks at 929 and 858 cm^{-1} corresponded to α-D-glucopyranose (Wang *et al.*, 2011; Jia *et al.*, 2021).

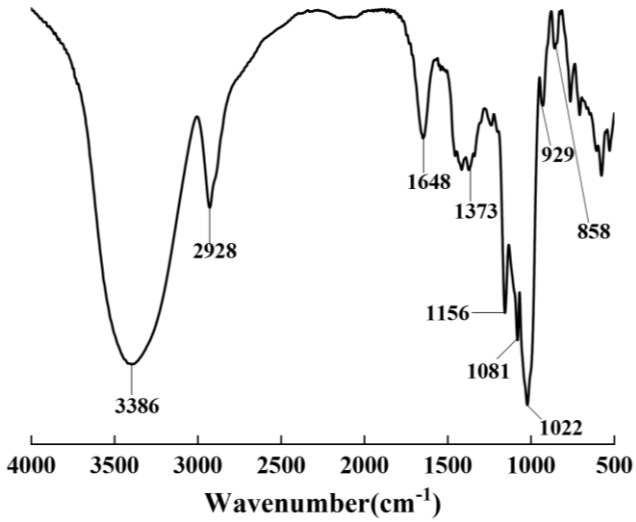

Figure 4. FT-IR spectrum of FYP.

Congo Red analysis

Congo red dye, upon mixing with polysaccharide containing a triple helical structure, showed a redshift in the maximum absorption wavelength (λ_{max}) compared to Congo Red dye alone with increasing NaOH solution concentration (Guo *et al.*, 2021). If the polysaccharide did not have a triple helical structure, the change trend of the λ_{max} of the Congo Red dye-polysaccharide complex was similar to that of the Congo Red dye alone. As shown in Figure 5, the λ_{max} of FYP combined with Congo Red exhibited a similar trend as the concentration of the NaOH solution increased from 0 to 0.6 mol/L, indicating that FYP had non-three helical structure. The λ_{max} of FYP was higher than that of the blank group, possibly because of the presence of hydrogen bonds and other bonds in FYP.

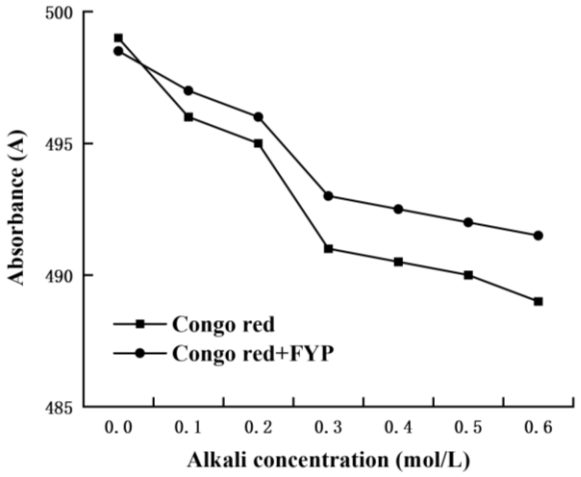

Figure 5. Congo Red absorption pattern of FYP

Antioxidant activity of FYP in vitro

DPPH is widely used to evaluate the free radical scavenging activity of natural extracts. Therefore, the DPPH free radical scavenging activity of FYP was estimated using V_C as the positive control. As shown in Figure 6A, FYP and V_C showed significant DPPH free radical scavenging ability in a concentration-dependent relationship in the range of 0.02 - 0.1 mg/mL. The DPPH free radical scavenging ability of FYP increased when the concentration increased from 0.02 to 0.1 mg/mL, and reached a maximum value (41.35%) at a concentration of 0.1 mg/mL, implying that FYP exhibited DPPH free radical scavenging ability.

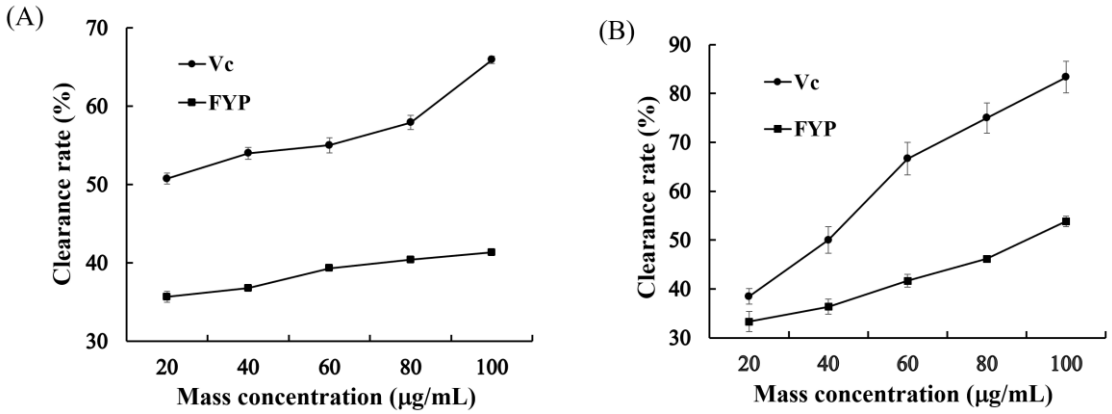

Figure 6. Effect of FYP on DPPH free radical scavenging rate **(A)** and superoxide anion free radical scavenging rate **(B)**.

The superoxide anion free radical is a highly toxic radical that can cause damage to the cells and organs, leading to diseases such as cancer and aging. Therefore, determining the superoxide anion free radical scavenging ability is an approach for evaluating the antioxidant activity. The superoxide anion free radical scavenging ability of FYP at different concentrations is displayed in Figure 6B. As shown in Figure 6B, FYP and V_C exhibited significant superoxide anion free radical scavenging ability in a concentration-dependent manner in the range of 0.02 - 0.1 mg/mL. The ability of FYP to scavenge superoxide anion free radicals gradually increased from 0.02 to 0.1 mg/mL, and the maximum scavenging rate (53.85%) of 0.1 mg/mL FYP was reached, which was lower than that of V_C, suggesting that FYP has a potential superoxide anion scavenging ability.

Conclusion

In the present work, the FYP extraction conditions were optimised by RSM. The optimal conditions were as follows: NaOH concentration of 1.0 mol/L, extraction time of 8 h, and solvent-to material-ratio of 10 mL/g. Under these conditions, the extraction yield of FYP was 64.52 ± 0.23%. FYP contained 91.70% total sugar with few proteins. FT-IR revealed that FYP exhibited absorption peaks characteristic of α-D-glucopyranose. Congo Red experiment showed that FYP had non-three helical structure. FYP exhibited good antioxidant activity against DPPH and superoxide anion free radicals.

Acknowledgement

The present work was financially supported by the Natural Science Fund of Hubei Province (grant no.: 2019CFB395), and Guiding Project of Scientific Research Plan of Hubei Provincial Department of Education (grant no.: B2020166).

References

Cheng, Z., Hu, M., Tao, J., Yao, H., Yan, P., An, G. and Wang, H. 2019. The protective effects of Chinese yam polysaccharide against obesity-induced insulin resistance. Journal of Functional Foods 55: 238-247.

Feng, X., Bie, N., Li, J., Zhang, M., Feng, Y., Ding, T., … and Wang, C. 2022. Effect of *in vitro* simulated gastrointestinal digestion on the antioxidant activity, molecular weight, and microstructure of polysaccharides from Chinese yam. International Journal of Biological Macromolecules 207: 873-882.

Guo, X., Kang, J., Xu, Z., Guo, Q., Zhang, L., Ning, H. and Cui, S. W. 2021. Triple-helix polysaccharides: Formation mechanisms and analytical methods. Carbohydrate Polymers 262: 117962.

Hao, L. X. 2016a. Immune activities of the water-soluble yam (*Dioscorea opposite* Thunb) polysaccharides as affected by thermal, acidic and enzymatic treatments. CyTA - Journal of Food 14: 1-5.

Hao, L. X. 2016b. Immunomodulatory potentials of the water-soluble yam (*Dioscorea opposita* Thunb) polysaccharides for the normal and cyclophosphamide-suppressed mice. Food and Agricultural Immunology 27: 667-677.

Hu, T., Cai, W., Cai, W., Zheng, Z., Xiao, Y. and Huang, Q. 2021a. Structure, size and aggregated morphology of a β-D-glucan from *Lignosus rhinocerotis* as affected by ultrasound. Carbohydrate Polymers 269: 118344.

Hu, T., Huang, Q., Wong, K. and Yang, H. 2017b. Structure, molecular conformation, and immunomodulatory activity of four polysaccharide fractions from *Lignosus rhinocerotis* sclerotia. International Journal of Biological Macromolecules 94: 423-430.

Hu, T., Huang, Q., Wong, K., Yang, H., Gan, J. and Li, Y. 2017a. A hyperbranched β-D-glucan with compact coil conformation from *Lignosus rhinocerotis* sclerotia. Food Chemistry 225: 267-275.

Hu, T., Wu, P., Zhan, J., Wang, W., Shen, J., Ho, C. T. and Li, S. 2021b. Influencing factors on the physicochemical characteristics of tea polysaccharides. Molecules 26(11): 3457.

Hu, T., Wu, P., Zhan, J., Wang, W., Shen, J., Wang, M., … and Li, S. 2022. Structure variety and its potential effects on biological activity of tea polysaccharides. Food Science and Human Wellness 11: 587-597.

Huang, H., Jiang, Q., Chen, Y., Li, X., Mao, X., Chen, X., … and Gao, W. 2016. Preparation, physico-chemical characterization and biological activities of two modified starches from yam (*Dioscorea opposita* Thunb). Food Hydrocolloids 55: 244-253.

Huang, R., Shen, M., Yu, Y., Liu, X. and Xie, J. 2020a. Physicochemical characterization and immunomodulatory activity of sulfated Chinese yam polysaccharide. International Journal of Biological Macromolecules 165: 635-644.

Huang, R., Xie, J., Yu, Y. and Shen, M. 2020b. Recent progress in the research of yam mucilage polysaccharides: Isolation, structure and bioactivities. International Journal of Biological Macromolecules 155: 1262-1269.

Jia, X., Wang, X., Liu, Y., Sun, Y., Ma, B., Li, Z. and Xu, C. 2021. Structural characterization of an alkali-extracted polysaccharide from *Dioscorea opposita* Thunb. with initial studies on its anti-inflammatory activity. Journal of Carbohydrate Chemistry 40: 308-324.

Ju, Y., Xue, Y., Huang, J., Zhai, Q. and Wang, X. H. 2014. Antioxidant Chinese yam polysaccharides and its pro-proliferative effect on endometrial epithelial cells. International Journal of Biological Macromolecules 66: 81-85.

Li, M., Chen, L. X., Chen, S. R., Deng, Y., Zhao, J., Wang, Y. and Li, S. P. 2017. Non-starch polysaccharide from Chinese yam activated RAW 264.7 macrophages through the Toll-like receptor 4 (TLR4)-NF-κB signaling pathway. Journal of Functional Foods 37: 491-500.

Li, P., Xiao, N., Zeng, L., Xiao, J., Huang, J., Xu, Y., … and Du, B. 2020. Structural characteristics of a mannoglucan isolated from Chinese yam and its treatment effects against gut microbiota dysbiosis and DSS-induced colitis in mice. Carbohydrate Polymers 250: 116-958.

Liu, X. X., Gu, L. B., Zhang, G. J., Liu, H. M., Zhang, Y. T. and Zhang, K. P. 2022. Structural characterization and antioxidant activity of polysaccharides extracted from Chinese yam by a cellulase-assisted method. *Process Biochemistry* 121: 178-187.

Liu, X. X., Yan, Y. Y., Liu, H. M., Wang, X. D. and Qin, G. Y. 2019. Emulsifying and structural properties of polysaccharides extracted from Chinese yam by an enzyme-assisted method. LWT - Food Science and Technology 111: 242-251.

Salehi, B., Sener, B., Kilic, M., Sharifi-Rad, J., Naz, R., Yousaf, Z., … and Santini, A. 2019. *Dioscorea* plants: A genus rich in vital nutra-pharmaceuticals - A review. Iranian Journal of Pharmaceutical Research 18(Suppl1): e124691.

Sun, Y., Wang, F., Liu, Y., An, Y., Chang, D., Wang, J., … and Cao, Y. 2022. Comparison of water- and alkali-extracted polysaccharides from Fuzhuan brick tea and their immunomodulatory effects *in vitro* and *in vivo*. Food and Function 13: 806-824.

Tang, Y., He, X., Liu, G., Wei, Z., Sheng, J., Sun, J., … and Yi, P. 2023. Effects of different extraction methods on the structural, antioxidant and hypoglycemic properties of red pitaya stem polysaccharide. Food Chemistry 405: 134804.

Wang, J., Ma, Z., Zhang, L., Fang, Y., Jiang, F. and Phillips, G. O. 2011. Structure and chain conformation of water-soluble heteropolysaccharides from *Ganoderma lucidum*. Carbohydrate Polymers 86: 844-851.

Wang, X., Huo, X. Z., Liu, Z., Yang, R. and Zeng, H. J. 2020. Investigations on the anti-aging activity of polysaccharides from Chinese yam and their regulation on klotho gene expression in mice. Journal of Molecular Structure 1208: 127895.

Wu, P., Tan, H., Zhan, J., Wang, W., Hu, T. and Li, S. 2020. Optimization of bioprocess extraction of *Poria cocos* polysaccharide (PCP) with *Aspergillus niger* β-glucanase and the evaluation of PCP antioxidant property. Molecules 25: 5930.

Yang, W., Wang, Y., Li, X. and Yu, P. 2015. Purification and structural characterization of Chinese yam polysaccharide and its activities. Carbohydrate Polymers 117: 1021-1027.

Zhang, Y., Li, S., Wang, X., Zhang, L. and Cheung, P. C. K. 2011. Advances in lentinan: Isolation, structure, chain conformation and bioactivities. Food Hydrocolloids 25: 196-206.

Zhou, S. 2021. Extraction, structural analysis, derivatization and antioxidant activity of polysaccharide from Chinese yam. Food Chemistry 361: 130089.