Original Research Paper

Preparation, Characterization and Antioxidant Activity of a Novel Acetylated Polysaccharide from *Flammulina Velutipes*

[1]Yingyun Peng, [1]Han Jiang, [1]Yufeng Wu, [1]Jinlong Zhang, [2]Jianhua Zhou and [1]Yiyong Chen

[1]*School of Biology and Food Engineering, Changshu Institute of Technology, China*
[2]*Center of Technology and Development, Zhangjiagang Tianyuan Ecological Breeding Co., Ltd, China*

Abstract: In this study, a novel polysaccharide FVP2a was extracted and purified from *F. velutipes.* Ac-FVP2a was prepared by chemical acetylation. The preparation process of Ac-FVP2a was optimized using the response surface method based on three experimental factors including reaction time, reaction temperature, and the ratio of FVP2a to acetic anhydride. The physicochemical characteristics and antioxidant activities of Ac-FVP2a *in vitro* were investigated. The results showed that the optimal preparation process of Ac-FVP2a was a reaction time of 2.5 h, reaction temperature of 45°C and ratio of FVP2a to acetic anhydride of 1:37 (g/mL), respectively. The monosaccharides of Ac-FVP2a with an average molecular weight of 1.97×10^5 Da were galactose, glucose, rhamnose, xylose, and arabinose and the molar ratio were 1.65:1:0.13:0.26:1.12. FT-IR suggested that Ac-FVP2a was prepared successfully. Ac-FVP2a had stronger antioxidant activities with better thermal stability than FVP2a. Ac-FVP2a might have the potential to become a novel antioxidant in the food industry.

Keywords: Polysaccharide, *Flammulina Velutipes*, Acetylation, Antioxidant Activity

Introduction

Growing evidence indicated that edible mushrooms had great potential to improve human health and prevent disease (Sitotaw *et al.*, 2020). Mushroom polysaccharides have become a field of attention and have good application prospects in food and medicine recently (Wang *et al.*, 2018) with antioxidant, immunomodulatory, anti-inflammatory, antihyperglycemic, antihyperlipidemic (Gao *et al.*, 2013; Liu *et al.*, 2013) activity. In addition, mushroom polysaccharides have the advantages of nontoxicity, local availability, and environmental friendliness, which are superior to synthetic antioxidants (Aug *et al.*, 2014). Bioactivities of some polysaccharides isolated from natural organisms show to be very weak and need to be further improved (Xie *et al.*, 2020). To improve the biological function of polysaccharides, effective ways need to be found. One of the effective ways to improve the biological activity of polysaccharides is chemical modification. In recent years, some polysaccharides which are chemically modified are more biologically active than their native polysaccharides (Liu *et al.*, 2012; Ma *et al.*, 2012). As one of the most commonly used chemical modification methods, acetylation is mainly used to modify the branched structure of polysaccharides (Xie *et al.*, 2020) using acetic

anhydride or acetyl chloride in suitable solvents (Endo *et al.*, 2015). The acetylated polysaccharide from *Ganoderma atrum* with appropriate Degree of Substitution (DS) had stronger antioxidant abilities (Chen *et al.*, 2014). The acetylated polysaccharides isolated from pumpkin exhibited higher antioxidant activity than that of unmodified polysaccharides (Song *et al.*, 2013). The emulsifying properties of acetylated polysaccharides from *Artemisia sphaerocephala Krasch.* seeds improved (Li *et al.*, 2016).

Flammulina velutipes is rich in amino acids, polysaccharides, vitamins, and fiber and is widely grown around the world such as in China and Japan (Jing *et al.*, 2014; Yang *et al.*, 2012). Polysaccharides from *F. Velutipes* (FVP) have various activities such as antitumor, immune regulation, anti-inflammatory, anti-aging, anti-oxidation, anti-hyperlipidemia, liver protection and improving memory, etc. (Pang *et al.*, 2007; Wang *et al.*, 2018; Shi *et al.*, 2012; Wu *et al.*, 2010; Zhang *et al.*, 2013). By reading a large number of literatures on FVP, we found that few investigations had focused on the acetylation modification process of FVP and its effects on bioactivity.

In this study, a new polysaccharide FVP2a from *F. velutipes* was isolated and purified. Based on a single factor experiment, Response Surface Methodology (RSM) was

used to optimize the preparation of Acetylated FVP2a (Ac-FVP2a) with the acetic anhydride method. The characterization of Ac-FVP2a was carried out by chemical composition analysis, UV-Vis, FT-IR, and thermogravimetric analysis. In addition, the *in vitro* antioxidant activity of Ac-FVP2a was also investigated compared with FVP 2a.

Materials and Methods

Materials

F. velutipes was purchased from Zhangjiagang Changxing Fungi Industry Co., Ltd. (Zhangjiagang, China). 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) was purchased from Shanghai Kaiyang Biotechnology Co., Ltd. (Shanghai, China). Anhydrous ethanol, acetic anhydride, sodium hydroxide, ferrous sulfate, hydrochloric acid, phenolphthalein, pyrogallol, potassium bromide, etc., are all analytical grades.

Preparation of FVP2a

The fresh *F. velutipes* were dried to less than 5% moisture and ground into powder. The powder (50 g) and distilled water (500 mL) were stirred and mixed in a beaker, and then extracted at 80°C for 8 h in a water bath. After the extraction step was complete, the supernatant was obtained by centrifuging (4000 rpm, 20 min) the extract at room temperature, which was then precipitated with four volumes of 95% ethanol at 4°C for 24 h. The precipitate was separated by centrifugation (20000 rpm, 5min) at 4°C and lyophilized to obtain crude FVP. The crude FVP was dissolved in distilled water and subjected to a DEAE-Sepharose CL-6B chromatography. FVP were fractionated by distilled water and eluted by stepwise aqueous sodium chloride solutions with concentrations of 0.1, 0.2, 0.5, and 1.0 mol/L at a flow rate of 1.0 mL/min, respectively. The four fractions FVP1, FVP2, FVP3, and FVP4 were obtained. The main fraction FVP2 was selected. FVP2 was further fractionated by Sephadex-G200 chromatography, which was eluted by stepwise aqueous sodium chloride solutions (0.15 mol/L) at a flow rate of 1.0 mL/min. A novel fraction of FVP2a was obtained due to its stronger antioxidant activity *in vitro*.

Preparation of Ac-FVP2a

The preparation of Ac-FVP2a was performed according to the previous method (Fu *et al.*, 2019). 10 mL distilled water was mixed with FVP2a (300 mg). The pH of the mixture was adjusted to 9.0 with NaOH solution (0.5 mol/L). Then the mixture was fully stirred for 4 h at a temperature of 30°C. While the mixture was stirring, the desired amount of acetic anhydride was added every 40 min. At the same time, the mixture and NaOH solution (0.5 mol/L) were mixed and stirred to keep the pH at 8.0-8.4. When the reaction was completed, the pH was adjusted to 7 with hydrochloric acid

(5 mol/L) to terminate the reaction. A dialysis membrane (molecular weight cut-off of 15,000 Da) was used to dialyze the reaction solution against deionized water for 48 h. Ethanol was added to the dialysate at 4°C for 24 h. The precipitate was lyophilized to obtain Ac-FVP 2a.

Analysis of Degree of Acetylation Substitution (DS)

Ac-FVP2a (10 mg) was added into 10 mL NaOH solution (0.01 mol/L). The mixed solution was added into 1-2 drops of phenolphthalein indicator. Then, HCl (0.01 mol/L) was used to titrate the solution. The disappearance of the red color indicated that the titration was complete. The volume of HCl was recorded. The calculation of DS was performed based on the following equation (Fu *et al.*, 2019):

$$A/\% = \frac{(V_0 C_0 - V_1 C_1) \times 0.043}{m} \times 100 \tag{1}$$

$$DS = \frac{162 \times A}{4300 - 42 \times A} \tag{2}$$

where, A is the acetyl group content (%). V_0 is the volume of NaOH solution (mL). V_1 is the volume of HCl. C_0 is the concentration of NaOH (mol/L). C_1 is the concentration of HCl (mol/L). m is the weight of Ac-FVP2a (g).

Experimental Design of RSM

According to the results of the preliminary single-factor experiment, Box-Benhnken's central combination experimental design was adopted to study the effect of reaction temperature (A), reaction time (B), and the ratio of FVP2a to acetic anhydride (C) on DS. The functional relationship between factors and response values was fitted by a designed three-factor and three-level regression equation. The preparation process of Ac-FVP2a was optimized using Design Expert software trial version 8.0 (Stat-Ease, Minneapolis) by RSM.

Chemical Composition Analysis of FVP2a and Ac-FVP2a

The phenol-sulfuric acid method was used to determine total carbohydrate content (Dubois *et al.*, 1956). The m-hydroxydiphenyl method was used to determine uronic acid content (Blumenkrantz and Asboe-Hansen, 1973). The protein content was determined by a reported method (Bradford, 1976).

Characterization of FVP2a and Ac-FVP2a

Analysis of Average Molecular Weight

Analysis of the average molecular weight of FVP2a and Ac-FVP2a was performed by high-performance gel permeation Chromatography. The chromatographic operating conditions were a mobile phase of 0.2 mol/L

sodium nitrate solution, a flow rate of 1 mL/min, and a column temperature of 45°C.

Analysis of Monosaccharide Composition

The monosaccharide composition analysis of FVP2a and Ac-FVP2a was performed by the gas chromatograph system (7820A GC, Agilent, USA) according to the previous method (Xie *et al.*, 2013). Xylose, mannose, ram nose, fucose, galactose, arabinose, and glucose were selected as standards.

Analysis of Ultraviolet-Visible Spectra

A dual-beam scanning UV-V is spectrophotometer was used to analyze the UV-Vis spectra of FVP2a and Ac-FVP2a in the range of 200-600 nm.

Analysis of FT-IR Spectra

FVP2a (1 mg) and Ac-FVP2a (1 mg) were dried and mixed with potassium bromide (10 mg). The mixture was pressed to obtain pellets for FT-IR spectra analysis by a fourier transform infrared spectrometer in the wavenumber range of 400 to 1000 cm^{-1}.

Thermogravimetric Analysis

A thermogravimetric analyzer was used to analyze the thermogravimetric of FVP2a and Ac-FVP 2a. FVP2a (1 mg) and Ac-FVP2a (1 mg) were placed in the sample pans, respectively. The operating conditions for thermogravimetric analysis were as follows: The temperature range was 100 to 800°C and the heating rate was 10°C/min.

Antioxidant Activities in Vitro of FVP2a and Ac-FVP2a

Assay for Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of FVP2a and Ac-FVP2a was performed based on the previous method (Yu *et al.*, 2019). First, different concentrations (0.1, 0.3, 0.5, 0.8 and 1.0 mg/mL) of FVP2a and Ac-FVP2a aqueous solutions were prepared.1 mL FVP2a and 1 mL Ac-FVP2a aqueous solution were mixed with 1 mL salicylic acid-ethanol (9 mmol/L), 1 mL hydrogen peroxide (8.8 mmol/L) and 1 mL ferrous sulphate (9 mmol/L), respectively. The mixture was incubated for 30 min at 37°C. The absorbance was determined at 510 nm. Distilled water was controlled. Vitamin C (V_c) was a positive control. The following equation was used to calculate the hydroxyl radical scavenging rate:

$$\text{Hydroxyl free radical scavenging rate} \atop (\%) = \left[1 - \left(A_i - A_j\right) / A_0\right] \times 100$$

where, A_i means the absorbance of the mixture. A_j means the absorbance of the mixture without salicylic acid-ethanol replaced by distilled water. A_0 means the absorbance of the mixture without the sample being replaced by distilled water.

Assay for DPPH Free Radical Scavenging Activity

An assay for DPPH radical scavenging activity was performed based on the reported method (Li and Shah, 2013). 2.0 mL of FVP2a and 2 mL of Ac-FVP2a aqueous solutions were added into 2 mL DPPH ethanol solution (25 mg/L), respectively. The mixture was incubated in the dark for 30min at room temperature before its spectroscopic analysis. The absorbance at 517 nm was determined. Vitamin C (V_c) was a positive control. The following equation was used to calculate DPPH radical scavenging rate:

$$\text{DPPH free radical scavenging rate} \atop (\%) = \left[A_0 - \left(A - A_b\right)\right] / A_0 \times 100$$

where, A_0 means the absorbance of DPPH solution with distilled water. A means the absorbance of samples with DPPH solution and A_b means the absorbance of samples with distilled water.

Assay for Superoxide Anion Radical Scavenging Activity

An assay for superoxide anion radical scavenging activity was carried out according to the previous report (Li and Shah, 2013). Briefly, 1.0 mL FVP2a and 1 mL Ac-FVP2a aqueous solution were added into Tris-HCl (4.5 mL, 50 mmol/L, pH 8.2) separately and the mixture was incubated at 25°C for 20 min. Then the mixture was mixed with 0.3 mL pyrogallol (3 mmol/L), which was shaken rapidly. OD_{325} value was determined every 30s. Vitamin C (V_c) was a positive control. The following equation was used to calculate the superoxide anion radical scavenging rate:

$$\text{Superoxide anion free radical scavenging} \atop rate(\%) = \left(1 - A / A_0\right) \times 100$$

where, A_0 means the absorbance of mixture solution with distilled water. A means the absorbance of samples with the reaction solution.

Reducing Power

The reducing power was performed based on the previous report (Chen and Huang, 2019a). FVP2a (1.0 mL) and Ac-FVP2a (1.0 mL) aqueous solution were mixed with 0.5 mL potassium ferricyanide solution (1%) and 0.2 mL PBS (pH 6.6, 0.2 mol/L). The mixture reacted at 50°C for 20 min. When the reaction ended, the reaction mixture was cooled to room temperature and then added to 0.2 mL ferric chloride solution (1%), 1 mL trichloroacetic acid solution (10%), and 3 mL distilled water and left for 10 min. Then, the absorption at 700 nm was determined.

Statistical Analysis

A design expert software was used to analyze the experimental data analysis of variance (ANOVA) program was used to analyze variance and significant differences. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

Model Building and Statistical Analysis

According to the principle of the Box-Behnken group and experimental design, a three-level regression equation of reaction temperature (A), reaction time (B), and ratio of FVP2a to acetic anhydride(C) was designed. The factor and level design are shown in Table 1. The response surface design and the results are shown in Table 2. Analysis of variance for the fitted quadratic polynomial model is shown in Table 3. The quadratic multinomial regression equation model was obtained as follows using multiple regression analysis:

$$Y=0.75+0.045A+0.094B-0.029C+0.073AB+0.077AC+0.0085BC+0.038A^2-0.00635B^2-0.069C^2$$

As shown in Table 3, the p-value (<0.05) and R^2 value (0.8859) of the established model were acceptable, indicating that the rationality of the established model and the preparation process of Ac-FVP2a can be described effectively by this model. The insignificant lack of fit ($F = 5.36$, $p = 0.0693$) suggested that other factors had little effect on DS. The results indicated that the model equation can be used to predict the preparation process of Ac-FVP2a effectively.

Preparation Process Optimization of Ac-FVP2a and Model Validation

Effect of cross-interaction of reaction time, reaction temperature, and the ratio of FVP2a to acetic anhydride on DS of Ac-FVP2a is presented in Fig. 1(A-C). The effect of both temperature and reaction time on DS of Ac-FVP2a was significant ($p<0.05$). Meanwhile, the effect of temperature and ratio of FVP2a to acetic anhydride on DS of Ac-FVP2a were significant ($p<0.05$). The effect of both reaction time and the ratio of FVP2a to acetic anhydride on DS of Ac-FVP2a was not obvious ($p>0.05$). Through regression model analysis, the optimal preparation process of Ac-FVP2a was as follows: reaction time was 2.53 h, the reaction temperature was 45.21°C and the ratio of FVP2a to acetic anhydride was 1:37.07 (g/mL). Under these conditions, the predicted DS value was 1.00689. FVP2a was acetylated to prepare Ac-FVP2a using the optimal process conditions for testing the reliability of response surface test results. For the feasibility of the experimental implementation, the predicted process

conditions were adjusted as follows: Reaction time was 2.5 h, the reaction temperature was 45°Cand the ratio of FVP2a to acetic anhydride was 1:37 (g/mL). Three parallel tests were carried out. The DS of Ac-FVP2a was 1.0065 close to the predicted value. From the analysis of the results, it could be concluded that the model was adequate for the preparation process of Ac-FVP2a.

Chemical Composition of FVP2a and Ac-FVP2a

The chemical composition of FVP2a and Ac-FVP2a is shown in Table 4. Ac-FVP2a contained 83.29±2.49% total carbohydrate and 17.15±1.36% uronic acid, which was higher than FVP2a. In addition, protein content was lower in Ac-FVP2a compared with FVP2a.

Characterization of FVP2a and Ac-FVP 2a

Monosaccharides and molecular weight of FVP2a and Ac-FVP2a is are seen in Table 5. The molecular weight of FVP2a and Ac-FVP2a was 2.95×10^4 and 1.97×10^5, respectively. The monosaccharides of FVP2a were galactose, glucose, rhamnose, xylose, and arabinose and the molar ratio were 1.65:1:0.13:0.26:1.12. The monosaccharides of Ac-FVP2a were galactose, glucose, rhamnose, xylose, and arabinose and the molar ratio were 1.65:1:0.13:0.26:1.12. The results showed that the acetylation modification could affect the composition and physicochemical characteristics of FVP2a.

The UV-Vis spectra of FVP2a and Ac-FVP2a are presented in Fig. 2A. The absorption of FVP2a and Ac-FVP2a around 260 or 280 nm was not significant, indicating a lower content of proteins and nucleic acids in FVP2a and Ac-FVP 2a. No apparent differences existed among FVP2a and Ac-FVP2a.

FT-IR spectra of FVP2a and Ac-FVP2a are presented in Fig. 2B. FVP2a and Ac-FVP2a had characteristic absorption peaks of polysaccharides. The broad and strong peak at 3409.92 cm^{-1} belonged to the stretching vibration of–OH. The narrow and weak peak at 2926.65 cm^{-1} was attributed to the stretching vibration of C-H (Chen and Huang, 2019b). The absorption peaks at 1639.49 and 1644.09 cm^{-1} suggested the existence of carboxyl groups (Chen and Huang 2019a). Compared with FVP 2a, the absorption peak at 1728.24 cm^{-1} of Ac-FVP2a belonged to the C = O stretching vibration, which indicated that FVP2a had been acetylated (Chen *et al.*, 2014; Ye *et al.*, 2021).

The thermogravimetric curve of FVP2a and Ac-FVP2a is presented in Fig. 2C.

The weights of FVP2a and Ac-FVP2a remained unchanged when the temperature was lower than 150°C. When the temperature exceeded 150°C, the loss of physically bound water led to a decrease in the weight of FVP2a and Ac-FVP 2a. When the temperature exceeded 200°C, the weight loss of FVP2a and Ac-FVP2a increased rapidly, which was because of the loss of chemically bound water. When the temperature exceeded 600°C, small changes occurred in weight loss.
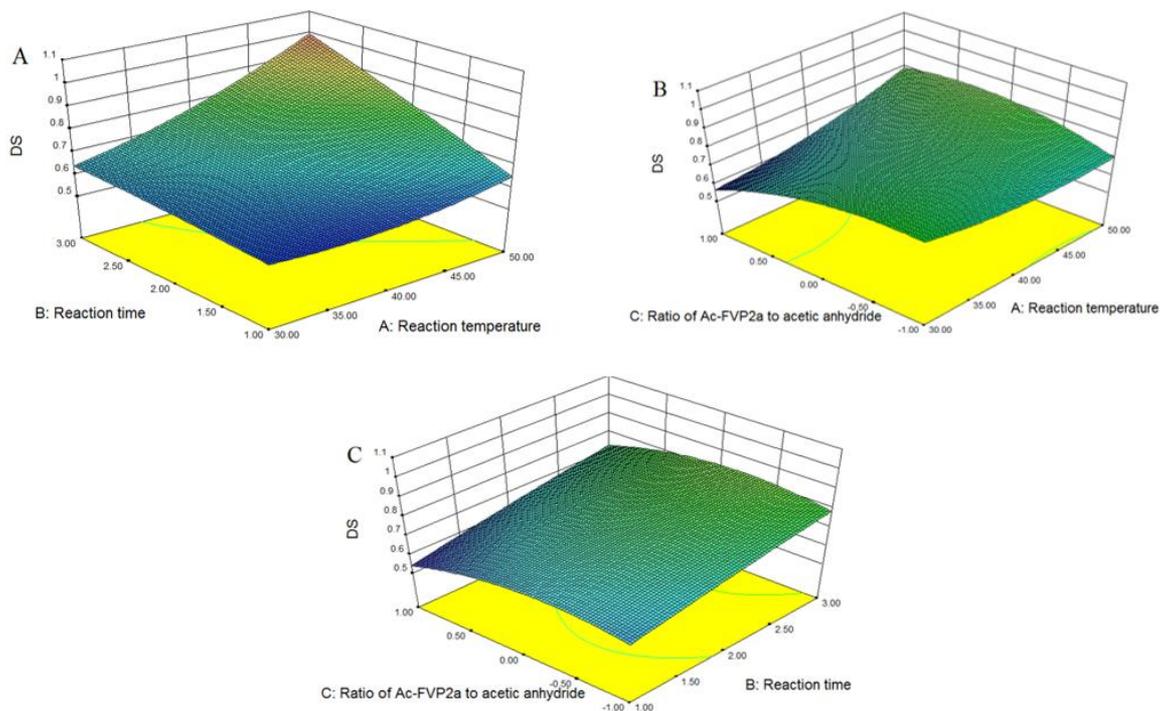
Fig. 1: Effect of cross-interaction of reaction temperature, reaction time, and the ratio of FVP2a to acetic anhydride on DS of Ac-FVP2a

Table 1: Factors and levels for response surface analysis

	Factors		
Level	Reaction temperature(A) /°C	Reaction time(B) /h	Ratio of Ac-FVP2a to acetic anhydride(C) /g/mL
-1	30	1.0	1:30
0	40	2.0	1:35
1	50	3.0	1:40

Table 2: Experimental design and results for response surface analysis

Runs	A	B	C	DS
1	0	1	-1	0.748
2	1	0	1	0.750
3	0	0	0	0.719
4	1	0	-1	0.729
5	0	-1	-1	0.585
6	-1	0	1	0.557
7	0	0	0	0.750
8	1	-1	0	0.681
9	-1	1	0	0.738
10	-1	-1	0	0.687
11	0	0	0	0.784
12	0	1	1	0.784
13	-1	0	-1	0.845
14	1	1	0	1.025
15	0	0	0	0.719
16	0	-1	1	0.587
17	0	0	0	0.784

However, Ac-FVP2a had slightly lower weight loss than FVP2a in the heating temperature range, which indicated that Ac-FVP2a had better thermal stability than FVP2a.

Antioxidant Activities of FVP2a and Ac-FVP2a

Antioxidant activities of FVP2a and Ac-FVP2a are presented in Fig. 3. The hydroxyl radical can react with any substance in the organism (Chen and Huang 2019b). The scavenging effect of FVP2a and Ac-FVP2a on hydroxyl free radicals was shown in Fig. 3(A). A positive correlation between the antioxidant activity of FVP2a and Ac-FVP2a and concentration was observed. The scavenging activity of Ac-FVP2a was higher than that of FVP2a. When the concentration was 1mg/mL, the scavenging rate of FVP2a and Ac-FVP2a was 51.2 and 72.6%, respectively.

DPPH radical is stable and can assess the free radical scavenging activity of antioxidants widely (Xie *et al.*, 2015). The scavenging effect of FVP2a and Ac-FVP2a on DPPH free radicals were concentration-dependent in Fig. 3(B). After acetylation modification, the scavenging activity of Ac-FVP2a on DPPH radicals improved than that of FVP2a to a certain extent.

When the concentration was 1.0 mg/mL, the scavenging rate of FVP2a and Ac-FVP2a was 57.2 and 67.6%, respectively. The results suggested that the addition of acetyl group to FVP2a could promote the DPPH free radical scavenging activity to a certain extent. The reason may be that the highly acetylated derivatives appear to be good hydrogen atom donors and can convert more radicals to stale products (Singh and Rajini, 2004)

Superoxide anion free radicals can attack biological macromolecules and disrupt cellular structure and function. Meanwhile, it is closely related to aging and pathological changes in organisms (Xie *et al.*, 2015). In Fig. 3(C), the scavenging activities of FVP2a and Ac-FVP2a on superoxide anion radicals were dose-dependent. When the concentration increased from 0.1 to 1.0 mg/mL, the scavenging effect of Ac-FVP2a was higher than that of FVP2a. When the concentration was 1 mg/mL, the scavenging rate of FVP2a and Ac-FVP2a was 51.2 and 72.6%, respectively. The antioxidant activities of polysaccharides improved after acetylation (Liu *et al.*, 2012; Ye *et al.*, 2021), which was consistent with our findings.
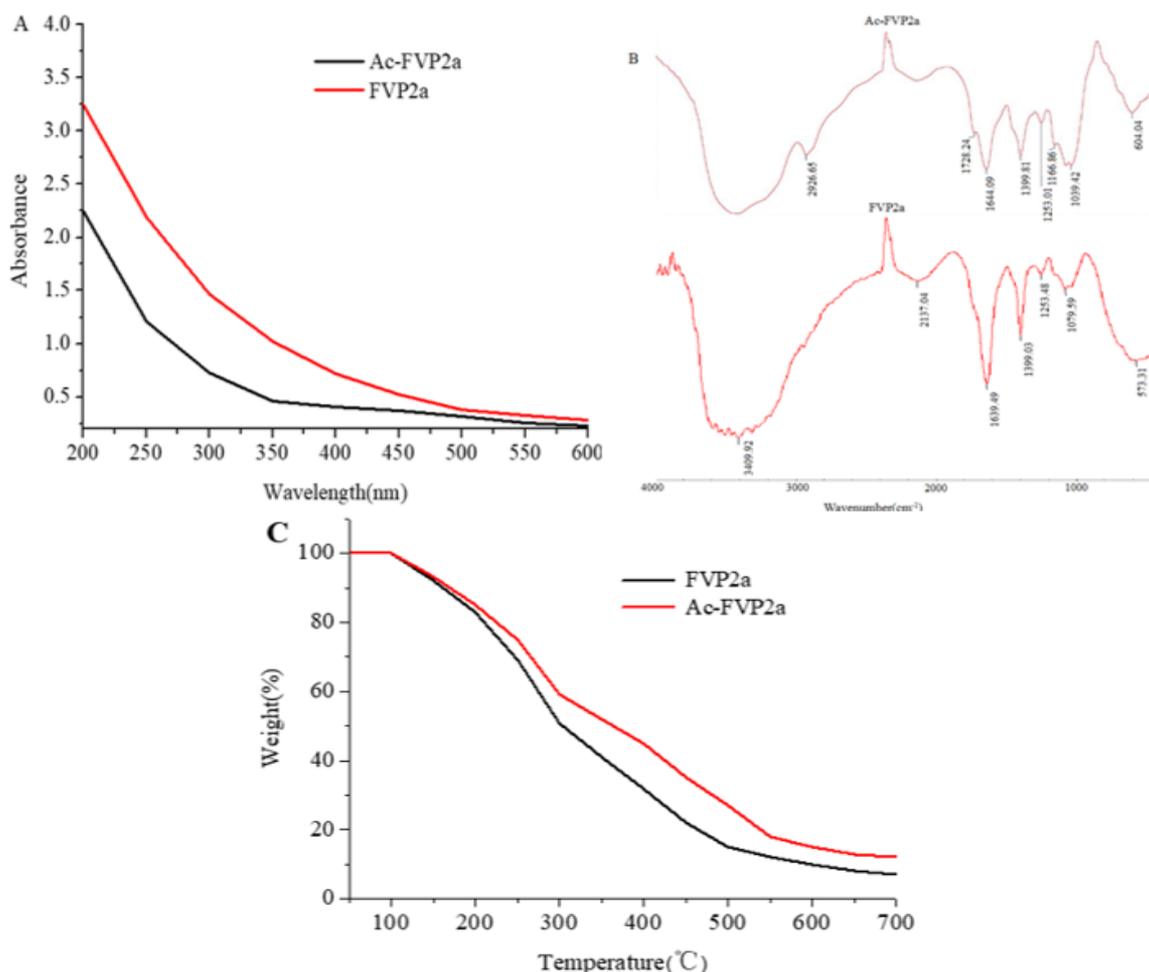

Fig. 2: Characterization of FVP2a and Ac-FVP2a (A: UV-Vis. B: FT-IR. C: Thermogravimetric)
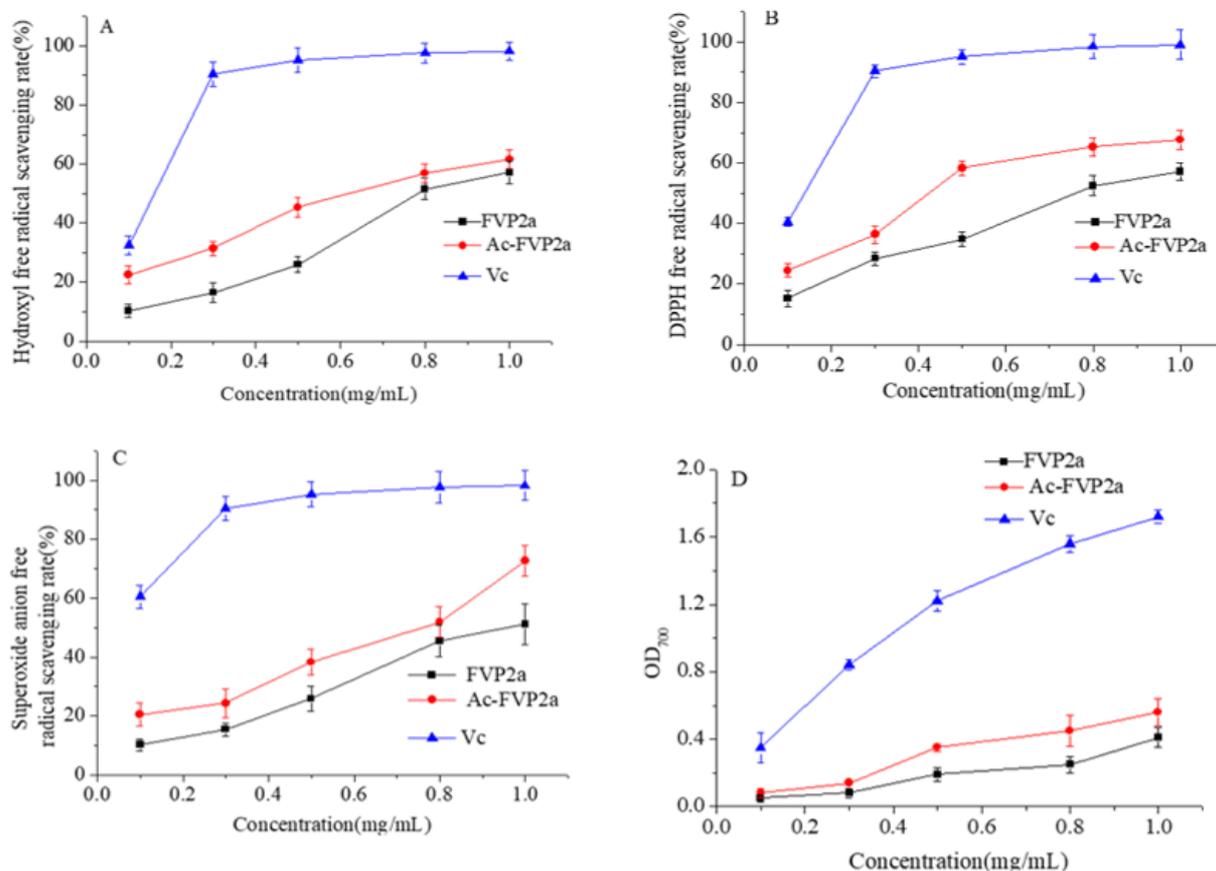
Fig. 3: Antioxidant activities *in vitro* of FVP2a and Ac-FVP2a. Hydroxyl radical (A), DPPH radical (B), Superoxide anion radical (C), and Reducing power (D)

Table 3: Analysis of variance for the fitted quadratic polynomial model

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	0.160000	9	0.018	6.040	0.0135*
A	0.016000	1	0.016	5.290	0.0550
B	0.071000	1	0.071	23.520	0.0019**
C	6.555×10^{-3}	1	6.555×10^{-3}	2.160	0.1848
AB	0.021000	1	0.021	7.080	0.0324*
AC	0.024000	1	0.024	7.880	0.0263*
BC	2.890×10^{-4}	1	2.890×10^{-4}	0.095	0.7664
A^2	6.048×10^{-3}	1	6.048×10^{-3}	2.000	0.2006
B^2	1.698×10^{-4}	1	1.698×10^{-4}	0.056	0.8197
C^2	0.020000	1	0.020	6.590	0.0372*
Residual	0.021000	7	3.030×10^{-3}		
Lackof fit	0.017000	3	5.661×10^{-3}	5.360	0.0693
Pure error	4.227×10^{-3}	4	1.057×10^{-3}		
Total	0.190000	16			
R^2	0.885900				

Notes: * means $p < 0.05$ (significant difference). ** means $p < 0.01$ (extremely significant difference).

Table 4: Chemical composition analysis of FVP2a and Ac-FVP2a

Sample	Uronic acid (%)	Protein (%)	Total carbohydrate (%)
FVP2a	13.29±1.76	2.23±0.18	79.25±2.37
Ac-FVP2a	17.15±1.36	1.16±0.26	83.29±2.49

Table 5: Monosaccharide and molecular weight of FVP2a and Ac-FVP2a

| Sample | Mw (Da) | Monosaccharide (molar ratio of monosaccharide, mol/ mol) | | | | |
		Galactose	Glucose	Rhamnose	Xylose	Arabinose
FVP2a	2.95×10^4	1.57	1	0.25	0.38	1.26
Ac-FVP2a	1.97×10^5	1.65	1	0.13	0.26	1.12

The reducing power of polysaccharides correlates with antioxidant activity (Liu *et al.*, 2018). When the sample concentration increased, the reducing power of FVP2a, Ac-FVP2a, and Vc increased as shown in Fig. 3(D). The reducing power of Vc was the strongest. Compared with FVP 2a, the reducing power of Ac-FVP2a was stronger. The reducing properties are always associated with reducing ketones. Reductone acts as an antioxidant by donating a hydrogen atom to disrupt the free radical chains. Our results revealed that acetylated polysaccharides tended to donate more electrons and terminate radical chain reactions, leading to a certain increase in their antioxidant activity. The introduction of acetyl groups into polysaccharides may affect the polarity, conformation, or charge density of native polysaccharides and weaken the dissociation energy of the O-H bond, which resulted in greater hydrogen donating capacity (Chen *et al.*, 2014).

Chemical modification of polysaccharides helps to obtain new antioxidants. Many studies have shown that the ability of polysaccharide derivatives to scavenge free radicals is mainly related to their chemical structure (Tsai *et al.*, 2007). Our results suggested that the antioxidant activity of FVP2a improved after acetylation. In addition, it is necessary to further study the effect of substitution degree on the antioxidant activity of Ac-FVP2a in the future.

Conclusion

In this study, a novel polysaccharide FVP2a was extracted and purified from *F. velutipes.* The preparation process ofAc-FVP2a was obtained by optimization using RSM. Characteristics and antioxidant activities *in vitro* of Ac-FVP2a were investigated. FT-IR suggested that Ac-FVP2a was prepared successfully. The results indicated that Ac-FVP2a had better thermal stability and stronger antioxidant activities than FVP2a. The acetylation could improve the antioxidant activity of FVP 2a and provide a promising preparation process for the acetylation of polysaccharides from *F. velutipes.* Ac-FVP2a might have the potential to become a novel antioxidant in the food industry. It is necessary to further study the structure and antioxidant mechanism of Ac-FVP2a in the future.

Acknowledgment

Author's Contributions

Yingyun Peng and Han Jiang: Research in all experiments and writing of the manuscript.

Yufeng Wu, Jinlong Zhang and Jianhua Zhou: Research on characterization and antioxidant activity of Ac-FVP2a and data processing.

Yiyong Chen: Project design, experimental guidance, and writing of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

References

Aug, A., Altraja, A., Altraja, S., Laaniste, L., Mahlapuu, R., Soomets, U., & Kilk, K. (2014). Alterations of bronchial epithelial metabolome by cigarette smoke are reversible by an antioxidant, O-methyl-L-tyrosinyl-γ-L-glutamyl-L-cysteinylglycine. American *Journal of Respiratory Cell and Molecular Biology*, 51(4), 586-594. http://doi.org/10.1165/rcmb.2013-0377OC

Blumenkrantz, N., & Asboe-Hansen, G. (1973). A new method for the quantitative determination of uronic acids. *Analytical Biochemistry*, 54(2), 484-489. http://doi.org/10.1016/0003-2697(73)90377-1

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. http://doi.org/10.1016/0003-2697(76)90527-3

Chen, F., & Huang, G. (2019a). Extraction, derivatization, and antioxidant activity of bitter gourd polysaccharide. *International Journal of Biological Macromolecules*, 141, 14-20. http://doi.org/10.1016/j.ijbiomac.2019.08.239

Chen, L., & Huang, G. (2019b). Antioxidant activities of phosphorylated pumpkin polysaccharide. *International Journal of Biological Macromolecules*, 125, 256-261. http://doi.org/10.1016/j.ijbiomac.2018.12.069

Chen, Y., Zhang, H., Wang, Y., Nie, S., Li, C., & Xie, M. (2014). Acetylation and carboxymethylation of the polysaccharide from Ganoderma atrum and their antioxidant and immunomodulating activities. *Food Chemistry*, 156, 279-288. http://doi.org/10.1016/j.foodchem.2014.01.111

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356. http://doi.org/10.1021/ac60111a017

Endo, R., Setoyama, M., Yamamoto, K., & Kadokawa, J. I. (2015). Acetylation of xanthan gum in ionic liquid. *Journal of Polymers and the Environment*, 23(2), 199-205. http://doi.org/10.1007/s10924-014-0701-8

Fu, Y., Yuan, Q., Lin, S., Liu, W., Du, G., Zhao, L., ... & Wu, D. T. (2019). Physicochemical characteristics and biological activities of polysaccharides from the leaves of different loquat (Eriobotrya japonica) cultivars. *International Journal of Biological Macromolecules*, 135, 274-281. http://doi.org/10.1016/j.ijbiomac.2019.05.157

Gao, C., Wang, Y., Wang, C., & Wang, Z. (2013). Antioxidant and immunological activity *in vitro* of polysaccharides from Gomphidius rutilus mycelium. *Carbohydrate Polymers*, 92(2), 2187-2192. http://doi.org/10.1016/j.carbpol.2012.12.011

Jing, P., Zhao, S. J., Lu, M. M., Cai, Z., Pang, J., & Song, L. H. (2014). Multiple-fingerprint analysis for investigating quality control of *Flammulina velutipes* fruiting body polysaccharides. *Journal of Agricultural and Food Chemistry*, 62(50), 12128-12133. http://doi.org/10.1021/jf504349r

Li, J., Hu, X., Li, X., & Ma, Z. (2016). Effects of acetylation on the emulsifying properties of Artemisia sphaerocephala Krasch. polysaccharide. *Carbohydrate Polymers*, 144, 531-540. http://doi.org/10.1016/j.carbpol.2016.02.039

Li, S., & Shah, N. P. (2013). Effects of various heat treatments on phenolic profiles and antioxidant activities of Pleurotus eryngii extracts. *Journal of Food Science*, 78(8), C1122-C1129. http://doi.org/10.1111/1750-3841.12189

Liu, J., Luo, J., Ye, H., & Zeng, X. (2012). Preparation, antioxidant and antitumor activities *in vitro* of different derivatives of levan from endophytic bacterium Paenibacillus polymyxa EJS-3. *Food and Chemical Toxicology*, 50(3-4), 767-772. http://doi.org/10.1016/j.fct.2011.11.016

Liu, Y., Huang, G., & Hu, J. (2018). Extraction, characterization, and antioxidant activity of polysaccharides from Chinese watermelon. *International Journal of Biological Macromolecules*, 111, 1304-1307. http://doi.org/10.1016/j.ijbiomac.2018.01.088

Liu, Y., Sun, J., Rao, S., Su, Y., & Yang, Y. (2013). Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from Catathelasma ventricosum in streptozotocin-induced diabetic mice. *Food and Chemical Toxicology*, 57, 39-45. http://doi.org/10.1016/j.fct.2013.03.001

Ma, L., Chen, H., Zhang, Y., Zhang, N., & Fu, L. (2012). Chemical modification and antioxidant activities of polysaccharide from mushroom Inonotus obliquus. *Carbohydrate Polymers*, 89(2), 371-378. http://doi.org/10.1016/j.carbpol.2012.03.016

Pang, X., Yao, W., Yang, X., Xie, C., Liu, D., Zhang, J., & Gao, X. (2007). Purification, characterization, and biological activity on hepatocytes of a polysaccharide from *Flammulina velutipes* mycelium. *Carbohydrate Polymers*, 70(3), 291-297. http://doi.org/10.1016/j.carbpol.2007.04.010

Shi, M., Yang, Y., Guan, D., Zhang, Y., & Zhang, Z. (2012). Bioactivity of the crude polysaccharides from fermented soybean curd residue by *Flammulina velutipes*. *Carbohydrate Polymers*, 89(4), 1268-1276. http://doi.org/10.1016/j.carbpol.2012.04.047

Singh, N., & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85(4), 611-616. http://doi.org/10.1016/j.foodchem.2003.07.003

Sitotaw, R., Lulekal, E., & Abate, D. (2020). Ethnomycological study of edible and medicinal mushrooms in Menge District, Asossa zone, Benshangul Gumuz region, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, 16(1), 1-14. http://doi.org/10.1186/s13002-020-00361-9

Song, Y., Yang, Y., Zhang, Y., Duan, L., Zhou, C., Ni, Y., ... & Hu, X. (2013). Effect of acetylation on the antioxidant and cytoprotective activity of polysaccharides isolated from pumpkin (Cucurbita pepo, lady godiva). *Carbohydrate Polymers*, 98(1), 686-691. http://doi.org/10.1016/j.carbpol.2013.06.049

Tsai, M. C., Song, T. Y., Shih, P. H., & Yen, G. C. (2007). Antioxidant properties of water-soluble polysaccharides from Antrodia cinnamomea in submerged culture. *Food Chemistry*, 104(3), 1115-1122. http://doi.org/10.1016/j.foodchem.2007.01.018

Wang, W. H., Zhang, J. S., Feng, T., Deng, J., Lin, C. C., Fan, H., ... & Jia, W. (2018). Structural elucidation of a polysaccharide from *Flammulina velutipes* and its immunomodulation activities on mouse B lymphocytes. *Scientific Reports*, 8(1), 1-13. http://doi.org/10.1038/s41598-018-21375-0

Wu, D. M., Duan, W. Q., Liu, Y., & Cen, Y. (2010). Anti-inflammatory effect of the polysaccharides of Golden needle mushroom in burned rats. *International Journal of Biological Macromolecules*, 46(1), 100-103. http://doi.org/10.1016/j.ijbiomac.2009.10.013

Xie, J. H., Liu, X., Shen, M. Y., Nie, S. P., Zhang, H., Li, C., ... & Xie, M. Y. (2013). Purification, physicochemical characterization, and anticancer activity of a polysaccharide from Cyclocarya paliurus leaves. *Food Chemistry*, 136(3-4), 1453-1460. http://doi.org/10.1016/j.foodchem.2012.09.078

Xie, J. H., Zhang, F., Wang, Z. J., Shen, M. Y., Nie, S. P., & Xie, M. Y. (2015). Preparation, characterization, and antioxidant activities of acetylated polysaccharides from Cyclocarya paliurus leaves. *Carbohydrate Polymers*, 133, 596-604.
http://doi.org/10.1016/j.carbpol.2015.07.031

Xie, L., Shen, M., Hong, Y., Ye, H., Huang, L., & Xie, J. (2020). Chemical modifications of polysaccharides and their anti-tumor activities. *Carbohydrate Polymers*, 229, 115436.
http://doi.org/10.1016/j.carbpol.2019.115436

Yang, W., Pei, F., Shi, Y., Zhao, L., Fang, Y., & Hu, Q. (2012). Purification, characterization, and anti-proliferation activity of polysaccharides from *Flammulina velutipes*. *Carbohydrate Polymers*, 88(2), 474-480. http://doi.org/10.1016/j.carbpol.2011.12.018

Ye, X., Zhao, Z., & Wang, W. (2021). Structural characterization and antioxidant activity of an acetylated Cyclocarya paliurus polysaccharide (Ac-CPP0. 1). *International Journal of Biological Macromolecules*, *171*, 112-122.
http://doi.org/10.1016/j.ijbiomac.2020.12.201

Yu, M., Chen, M., Gui, J., Huang, S., Liu, Y., Shentu, H., ... & Zhang, Y. (2019). Preparation of Chlorella vulgaris polysaccharides and their antioxidant activity *in vitro* and *in vivo*. *International Journal of Biological Macromolecules*, 137, 139-150.
http://doi.org/10.1016/j.ijbiomac.2019.06.222

Zhang, Z., Lv, G., He, W., Shi, L., Pan, H., & Fan, L. (2013). Effects of extraction methods on the antioxidant activities of polysaccharides obtained from *Flammulina velutipes*. *Carbohydrate Polymers*, 98(2), 1524-1531.
http://doi.org/10.1016/j.carbpol.2013.07.052