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Phosphorylation, Characterization, and Antioxidant Activity of Polysaccharides from *Flammulina Velutipes* Scraps

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Abstract: In this study, polysaccharides from *Flammulina velutipes* Scraps (FVSP) were extracted. The preparation conditions of the Phosphorylated derivative of FVSP (Ph-FVSP) were optimized based on single experiments and response surface analysis. Characterization and antioxidant properties of FVSP and Ph-FVSP were investigated. The optimum phosphorylation modification process of FVSP obtained was as follows: The mass ratio of STPP/STMP was 1:2, the reaction temperature was 70°C, the reaction time was 5.4 h and the pH was 9.2. Infrared spectroscopy suggested that the phosphorylation of FVSP was successful. Compared with FVSP, Ph-FVSP was more capable of scavenging hydroxyl radicals and superoxide anions. Our results revealed that Ph-FVSP had potential as a novel food additive and antioxidant in the food industry.

Keywords: Polysaccharide, *Flammulina Velutipes* Scraps, Phosphorylation, Response Surface Methodology (RSM), Antioxidant Activity

Introduction

Polysaccharides are composed of more than 10 monosaccharide molecules and are widely found in nature. Polysaccharides exist mainly in the form of glycogen in animals and in the form of cellulose, peptidoglycan, and starch in plant cells (Xie *et al*., 2016). Many microorganisms can also produce polysaccharides through metabolism (Li and Shah, 2014). Polysaccharides can be used in many fields due to their biological activities such as antioxidant (Chen *et al*., 2020a), antitumor (Ji *et al*., 2020; Xie *et al*., 2013), antiviral (Hajji *et al*., 2019), hypoglycemic (Chen *et al*., 2020b), anti-inflammatory (Hou *et al*., 2020) and so on. Modified polysaccharides tend to exhibit better activity than their native state. Chen *et al*. (2015) found that sulfated Ganoderma lucidum polysaccharides enhanced the stimulation of TNF-α protein secretion, a major pro-inflammatory cytokine produced by macrophages (Chen *et al*., 2015). Wang *et al*. (2018a) studied carboxymethylated and sulfated derivatives of an exopolysaccharide (LEP) from the fermented broth of *Lachnum* YM240. They found that carboxymethylated polysaccharides were higher than LEP in terms of the scavenging capacity of DPPH radicals and hydroxyl radicals. Furthermore, carboxymethylated-LEP and sulfated-LEP had a better inhibitory effect on the enzyme activity of α-glucosidase and α-amylase and the diffusion of glucose than LEP (Wang *et al*., 2018b). Du *et al*. (2018) investigated the antioxidant activities of acetylated derivative TAPA1-ac of polysaccharide from *Tremella* aurantialba and found that acetylation could significantly improve the antioxidant activities and oxidative injury protection effects (Du *et al*., 2018). Selenization of lily polysaccharides could significantly enhance the immune-enhancing activity of chicken (Hou *et al*., 2016).

Phosphorylation is a kind of method used for the chemical modification of polysaccharides. Like other modified polysaccharides, phosphorylation can also significantly change the biological activities of polysaccharides (Xia *et al*., 2021). Many phosphorylation reagents were applied in polysaccharide modification. Phosphorylated β-D-glucan was prepared with phosphoric acid. Phosphorylated polysaccharide with a degree of substitution of 0.153 was easy to degrade due to the high temperature and high acidity (Chen *et al*., 2009). Phosphorus oxychloride was another phosphorylation reagent. The polysaccharide was added to N, N-dimethylformamide, and stirred for a long time at room temperature. Then, phosphorus oxychloride was slowly added to the solution and reacted at a specified temperature and time, after purification, phosphorylated polysaccharide was obtained (Chen *et al*., 2021). This reaction method could produce phosphorylated polysaccharides with a high degree of substitution in a short time but had more toxic by-products. Phosphorus pentoxide was usually used to prepare phosphorylated chitosan. Chitosan and phosphorus pentoxide were dissolved in methanesulfonic acid and reacted in an ice-water bath under an inert atmosphere of N_2

to produce phosphorylated chitosan (Hu *et al.*, 2012). Phosphates were common phosphorylation reagents for their low price including disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium tripolyphosphate, sodium trimetaphosphate, etc. Reaction conditions (temperature, time, and pH) and the proportion of reagents could significantly influence the degree of substitution (Ahmad, 2021).

Flammulina velutipes scraps are the waste after the edible parts of *Flammulina velutipes* are removed. *F. velutipes* Scraps Polysaccharide (FVSP) is mainly composed of glucose, mannose, galactose, fucose, and arabinose (Wang and Zhang, 2021). As a kind of fungus polysaccharide, its physiological and biochemical activity had been reported in many types of research (Fukushima *et al.*, 2001; Zhao *et al.*, 2019; Lin *et al.*, 2016). However, there is no report on the phosphorylation modification of FVSP and the antioxidant activity of Phosphorylated FVSP (Ph-FVSP).

Therefore, in this study, the phosphorylation modification process of FVSP was optimized based on a single factor experiment and response surface methodology. Fourier Transform Infrared spectroscopy (FT-IR) was used to preliminarily characterize Ph-FVSP. The antioxidant activity of Ph-FVSP and FVSP *in vitro* was also investigated.

Materials and Methods

Materials

F. velutipes scraps were provided by an edible fungus farm in Changshu, Jiangsu province, China. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) was provided by Sigma Aldrich (Sigma, St Louis, MO, USA). Anhydrous ethanol, trichloromethane, Sodium Trimetaphosphate (STMP), Sodium Tripolyphosphate (STPP), sodium sulfate, concentrated sulfuric acid, concentrated nitric acid, hydrochloric acid, hydrogen peroxide, N-butanol, ascorbic acid, potassium dihydrogen phosphate, salicylic acid, ferrous sulfate, pyrogallol, and other reagents were of analytical grade.

Preparation of FVSP

After removing impurities, *F. velutipes* scraps were dried at 60°C and ground into powder. The powder was extracted with water at 80°C for 160 min. The extract was centrifuged at 5000 rpm at 20°C for 10 min. The supernatant was concentrated to one-fifth volume. Sevag regent (n-butanol/trichloromethane = 1:5) was used to remove the protein. Then the solution was concentrated by vacuum evaporation and precipitated using 95% ethanol. FVSPs were prepared by recovering the pellet by centrifugation at 10,000 rpm for 10 min at 20°C and freeze-drying.

Phosphorylation of FVSP

The phosphorylation of FVSP was performed mainly according to the reported method with a small modification (Ye *et al.*, 2013). Phosphorylation reagents (7 g) (STPP and STMP, in a suitable mass ratio) were dissolved in distilled water (100 mL). FVSP (1 g) and sodium sulfate (5 g) were mixed with the phosphorylation solution. The pH was adjusted to 9.0. Then the reaction solution was kept at 60°C for at least 2 h. After the reaction, the solution was precipitated by anhydrous ethanol for 24 h and centrifuged at 5000 r/min for 10 min. The precipitation was dialyzed against deionized water and lyophilized to obtain Ph- FVSP.

Determination of Phosphate Content of Ph- FVSP

The determination of phosphate content of Ph- FVSP was performed by molybdenum blue colorimetry (Zhang *et al*., 2017). Concentrated nitric acid (1 mL) and concentrated sulfuric acid (1 mL) were successively added to a beaker with Ph-FVSP (0.5 g) in it. The mixed sample was heated until white smoke emerged. When the mixed sample was cooled, 1 mL of 30% hydrogen peroxide was mixed with it. The sample was then heated again until white smoke emerged. The above operation was repeated until no smoke was emitted to ensure the complete decomposition of Ph-FVSP. After cooling, the sample was added to hydrochloric acid (1 mL, 6 mol/L) and heated again. At last, the sample was transferred to a volumetric flask and diluted with deionized water to 100 mL. 5 mL sample was mixed with 3 mL Tris buffer (1 mol/L), 1 mL ascorbic acid solution (20%), 1 mL sulphuric acid solution (3 mol/L), and 1 mL molybdic acid solution (3%). The mixture was shaken well and kept at 30°C for 30 min. The absorbance of the mixture at 580nm was determined. Phosphate content was calculated from calibration curve using potassium dihydrogen phosphate standard solution ($Y = 0.085X - 0.0037$，$R^2 = 0.9988$):

$$Phosphate\ content(\%) = C_1V_1/m_1 \times 100 - C_0V_0/m_0 \times 100$$

where, C_1 and C_0 were phosphate contents calculated from the standard curve of FVSP and Ph-FVSP, respectively. V_1 and V_0 were dilution volumes of 100 mL, *and* m_1 and m_0 were the weight of FVSP and Ph-FVSP, respectively (0.5 g).

Effect of Mass Ratio of STPP/STMP on the Phosphate Content of Ph-FVSP

According to the preparation method of Ph-FVSP as above mentioned. The mass ratio of STPP/STMP was 0:7, 1:6, 2:5, 3:4, 4:3, 5:2, 6:1 and 7:0, respectively. The pH was adjusted to 9.0. The reaction system was kept at 60°C for 3 h. The effect of the mass ratio of STPP/STMP on the phosphate content of Ph-FVSP was investigated by the determination of the phosphate content of Ph-FVSP.

Effect of Reaction Temperature on the Phosphate Content of Ph-FVSP

In the reaction system, the mass ratio of STPP/STMP was 3:4. The pH was adjusted to 9.0. The reaction was

kept at 40, 50, 60, 70, and 80℃ for 3 h, respectively. The effect of reaction temperature on the phosphate content of Ph-FVSP was investigated by the determination of the phosphate content of Ph-FVSP.

Effect of Reaction Time on the Phosphate Content of Ph-FVSP

In the reaction system, the mass ratio of STPP/STMP was 3:4. The pH was adjusted to 9.0. The reaction system was kept at 60℃ for 2, 3, 4, 5, and 6 h, respectively. The effect of reaction time on the phosphate content of Ph-FVSP was investigated by the determination of the phosphate content of Ph-FVSP.

Effect of pH on the Phosphate Content of Ph-FVSP

In the reaction system, the mass ratio of STPP/STMP was 3:4. The pH was adjusted to 7.0, 8.0, 9.0, 10.0, and 11.0, respectively. The reaction system was kept at 60℃ for 3 h. The effect of pH on the phosphate content of Ph-FVSP was investigated by the determination of the phosphate content of Ph-FVSP.

*Experimental Design of Response Surface Method*s

Design Expert software trial version 8.0 (Stat-Ease, Minneapolis) was used for experimental design and statistical analysis. Box-Behnken Design (BBD) was used to assess the effect of the mass ratio of STPP/STMP (*A*), reaction temperature (*B*), reaction time (*C*), and pH (*D*), which were prescribed into three levels, coded +1, 0 and -1 for high, intermediate and low value, respectively. The phosphate content of Ph-FVSP was set as the response. The independent variables and levels of the experimental design were shown in Table 1.

Characterization of Ph-FVSP

KBr (spectroscopic grade) was mixed with dried FVSP and Ph-FVSP, respectively. After grinding and pressing the mixture, infrared spectroscopic analysis was performed using a Nicolet 6700 FT-IR spectrometer (Thermo, USA) in the frequency range of 400-4000 cm^{-1} (Fu *et al.*, 2019).

Assay for Hydroxyl Radical Scavenging Activity

An assay for hydroxyl radical scavenging activity of FVSP and Ph-FVSP was performed according to the reported method (Yu *et al.*, 2019). 1 mL FVSP and Ph-FVSP aqueous solution (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) was mixed with 1 mL salicylic acid-ethanol (9 mmol/L), 1 mL ferrous sulfate (9 mmol/L) and 1 mL hydrogen peroxide (8.8 mmol/L), respectively. After standing at 37℃ for 30 min, the absorbance at 510 nm was measured. Distilled water was used as a control. The hydroxyl radical scavenging activity was calculated based on the following formula:

$$\textit{Hydroxyl radical scavenging activity}(\%) = \left[1-\left(A_i - A_j\right)/A_0\right]\times 100.$$

Where, A_i was the absorbance of samples. A_j was the absorbance of samples after replacing salicylic acid-ethanol with distilled water. A_0 was the absorbance of samples after replacing FVSP or Ph-FVSP with distilled water.

Assay for DPPH free Radical Scavenging Activity

An assay for DPPH radical scavenging activity was performed according to the reported method (Li and Shah, 2013). 25 mg/L of DPPH was prepared in absolute ethanol. 2 mL DPPH solution was mixed with 2.0 mL FVSP and Ph-FVSP aqueous solution (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL), respectively. After keeping in dark for 30 min at room temperature, the absorbance of the mixture at 517 nm was measured. The DPPH radical scavenging activity was determined according to the following equation:

$$\textit{DPPH radical scavenging activity}(\%) = \left[A_0 - \left(A - A_b\right)\right]/A_0 \times 100.$$

Where, A was the absorbance of the mixture; A_0 was the absorbance of the mixture after replacing FVSP or Ph-FVSP with distilled water; A_b was the absorbance of the mixture after replacing DPPH solution with distilled water.

Assay for Superoxide Anion Radical Scavenging Activity

The Pyrogallol autoxidation method was used to determine the superoxide anion radical scavenging activity (Li and Shah, 2013). 3 mmol/L of pyrogallol was prepared with 10 mmol/L HCl as solvent. 1.0 mL FVSP and Ph-FVSP aqueous solution (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) were mixed with 4.5 mL Tris-HCl (50 mmol/L, pH 8.2) respectively and kept at 25℃ for 20 min. Then 0.3 mL pyrogallol solution was added to the mixture and shaken rapidly. The absorbance of the mixture was measured at 325 nm every 30 s. Superoxide anion radical scavenging activity was calculated using the following equation:

$$\textit{Superoxide anion radical scavenging activity}(\%) = \left(1 - A/A_0\right)\times 100$$

Where, A_0 was the absorbance of mixture solution after replacing FVSP or Ph-FVSP with distilled water. A was the absorbance of the mixture solution.

Table 1: Independent variables and levels of the experimental design

Independent variable	levels		
	-1.0	0.0	1.0
A: Mass ratio of STPP/STMP	1:6	2:5	3:4
B: Reaction temperature (℃)	60.0	70.0	80.0
C: Reaction time (h)	4.0	5.0	6.0
D: pH	8.0	9.0	10.0

Results and Discussion

Effect of Mass Ratio of STPP/STMP on the Phosphate Content of Ph-FVSP

The effect of the mass ratio of STPP/STMP on the phosphate content of Ph-FVSP was shown in Fig. 1(A). As shown in Fig. 1(A), when the mass ratio of STPP/STMP changed from 0:7 to 3:4, the content of phosphate in Ph-FVSP increased and reached the maximum value of 6.82±0.19%. Then, when the proportion of STPP in phosphorylation reagent increased, the content of phosphate showed a downward trend.

Effect of Reaction Temperature on the Phosphate Content of Ph-FVSP

The effect of reaction temperature on the phosphate content of Ph-FVSP was shown in Fig. 1(B). When the temperature rose from 40 to 70℃, the phosphate content increased gradually and up to a peak value of 7.13±0.11%. When the reaction acted at 80℃, the phosphate content declined, which might be due to the dissociation of Ph-FVSP.

Effect of Reaction Time on the Phosphate Content of Ph-FVSP

The effect of reaction time on the phosphate content of Ph-FVSP was shown in Fig. 1(C). From 2 to 3 h, the content of phosphate in Ph-FVSP increased rapidly. From 3 to 5 h, the content of phosphate increased slowly and reached a maximum value of (7.25±0.12) %. After 5 h of reaction time, the content of phosphate decreased, which might be due to the dissociation of Ph-FVSP.

Effect of pH on the Phosphate Content of Ph-FVSP

Figure 1(D) indicated the effect of pH on the phosphorylation content of Ph-FVSP. It could be concluded that an alkaline environment was more favorable for the phosphorylation of Ph-FVSP. The reason might be that glycosidic bonds were easier to degrade under acidic conditions (Chen and Huang, 2018; Muhammad *et al.*, 2000). When pH was adjusted from 7 to 9, the content of phosphate in Ph-FVSP increased up to a peak value of 6.74±0.18%. When pH was adjusted to 11, the content of phosphate declined, which implied that excessively alkaline could inhibit phosphorylation.

Response Surface Analysis

According to the results of the single factor experiment, Box-Behnken design software was used to further optimize the phosphorylation process of Ph-FVSP by response surface methodology. 29 experiments were carried out to optimize the four individual parameters. The value of independent process responses (*A*, *B*, *C*, and *D*) and the content of phosphate in Ph-FVSP was shown in Table 2. Based on the multiple regression analysis, the response variable and the tested variables were related by the following second-order polynomial equation:

$$Y = 7.52 + 0.071 \times A - 0.007 \times B + 0.19 \times C + 0.13 \times D + 0.022$$
$$Y = 7.52 + 0.071 \times A - 0.007 \times B + 0.19 \times C + 0.13 \times D + 0.022$$
$$0.085 \times C \times D - 0.53 \times A^2 - 0.58 \times B^2 - 0.022 \times C^2 - 0.36D^2$$

The analysis of variance for the fitted quadratic polynomial model was shown in Table 3. The F-value of the model was 25.99 and the p-value was lower than 0.0001, which indicated a more significant effect on the response variable. The lack of fit was not significant (F = 0.92, p = 0.5877), which indicated that the model equation was appropriate to predict the content of phosphate in Ph-FVSP. The variables *C*, *D*, *AD*, *BD*, A^2, B^2, C^2, and D^2 had a very significant effect on the yield of Ph-FVSP (P<0.01). By comparing the first-order coefficients of the regression equation, the influence of each factor on the phosphorylation of Ph-FVSP was as follows: *C*(reaction time)>*D*(pH)>*A*(the mass ratio of STPP/STMP)>*B*(reaction temperature).

The correlation coefficient (R^2 = 0.9629) indicated that the total variation of 96.29% was produced by the independent variables. The adjusted correlation coefficient (R_{adj}^2 = 0.9259) and the CV value (C.V.% = 1.71%) showed that the model had a high fitting degree and high precision. The results suggested that the regression equation could be used to choose the best phosphorylation process conditions and predict the experimental results.

Optimization of Phosphorylation Process of Ph-FVSP

The 3D response surface of the effects of the mass ratio of STPP/STMP, the reaction temperature, the reaction time, the pH, and their interactions on the phosphorylation rate of Ph-FVSP were shown in Fig. 2(A-F). In Fig. 2(A), the contour plot was close to a circle, which implied that the interaction of the mass ratio of STPP/STMP and the temperature was not significant. In Fig. 2(B), the response surface curve for a time was steeper than that for the mass ratio of STPP/STMP, which indicated that there was an interaction between the two variables and the effect of time on the phosphorylation rate of Ph-FVSP was more significant. Figure 2(C) displayed the effects of the mass ratio of STPP/STMP, the pH, and their interactions on the phosphorylation rate of Ph-FVSP, which indicated that the pH on the phosphorylation rate was more significant. The variable of time on the phosphorylation rate was more significant compared with temperature as shown in Fig. 2(D). Figure 2(E) indicated that the effect of pH was greater than the temperature in the interaction on the phosphorylation rate of Ph-FVSP. Figure 2(F) showed that pH had a more important effect than time on the phosphorylation rate.

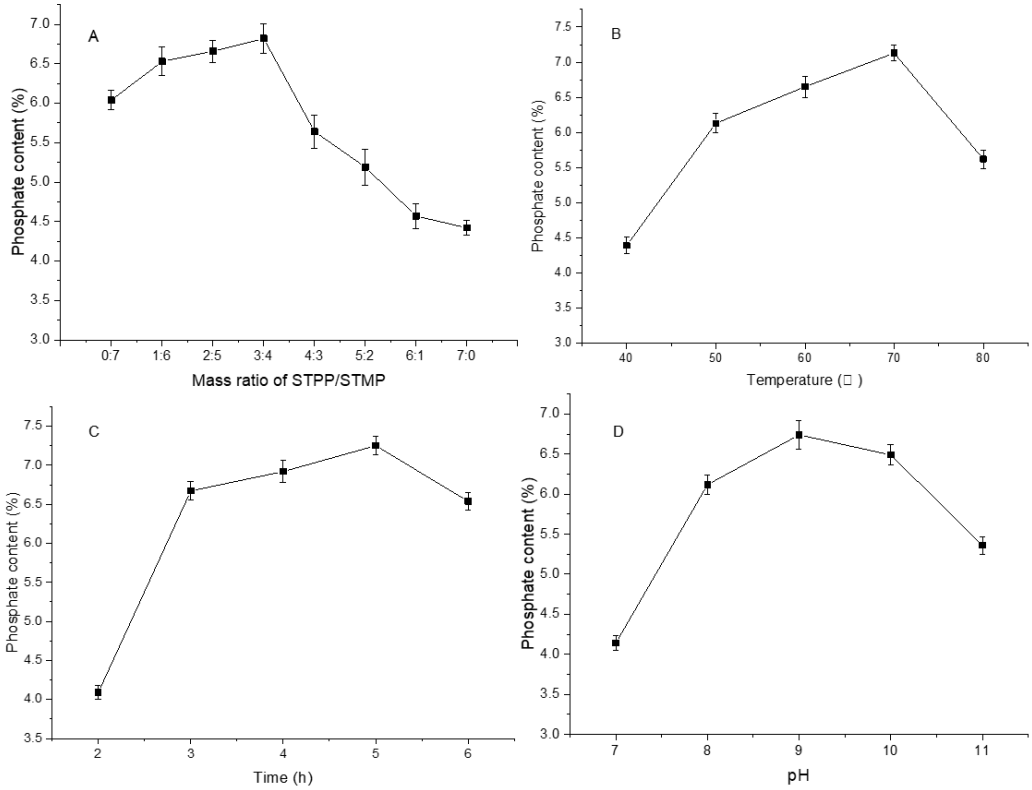
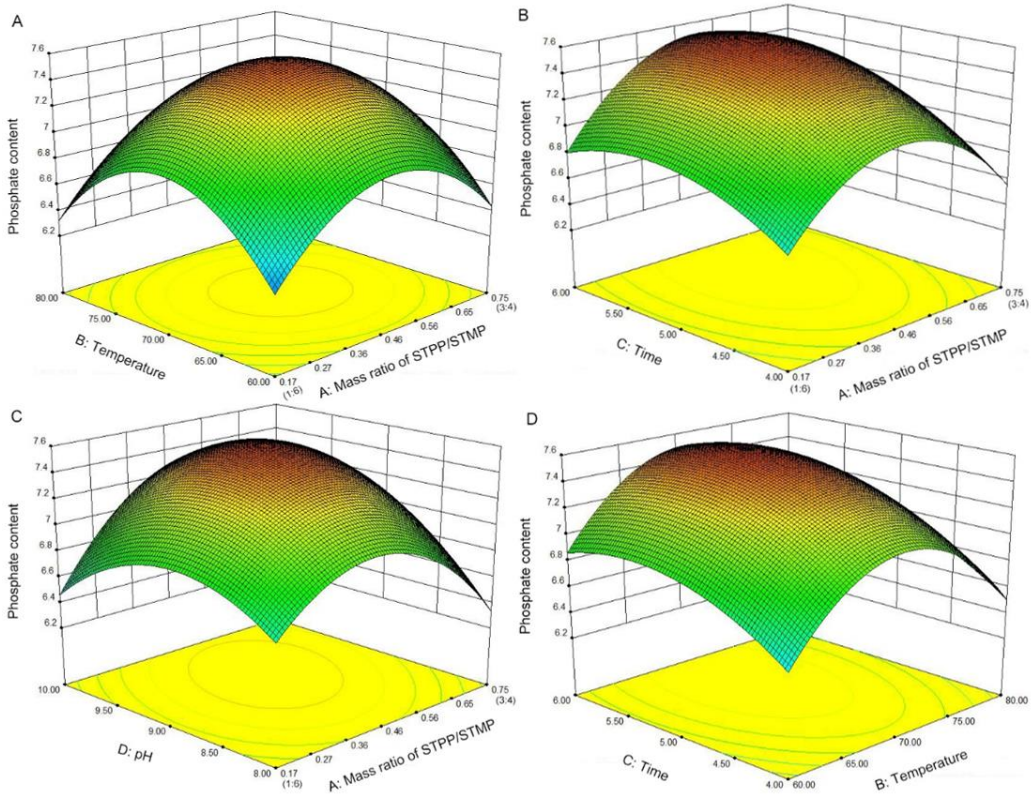


Fig. 1: Effect of the mass ratio of STPP/STMP (A), reaction temperature (B), reaction time (C), and pH (D) on the phosphorylation of Ph-FVSP



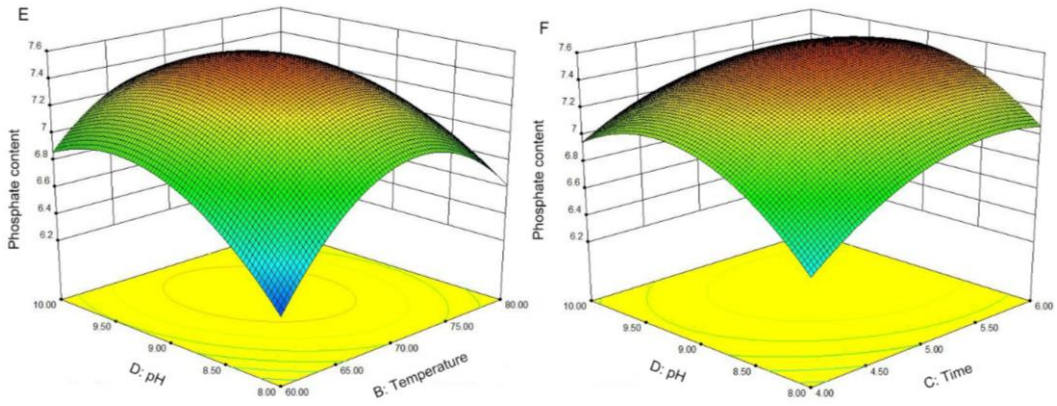


Fig. 2: Response surface plots showing the effects of the mass ratio of STPP/STMP, the reaction temperature, the reaction time, and the pH on the content of phosphate in

Based on the regression model analysis, the optimal phosphorylation condition of FVSP phosphorylation was the mass ratio of STPP/STMP of 1:2, reaction temperature of 69.95℃, and reaction time of 5.41 h, and pH of 9.18. Under the optimal phosphorylation condition, the predicted value of the content of phosphate was 7.59%. To easily operate, the optimal parameters were conducted to be the mass ratio of STPP/STMP of 1:2, reaction temperature of 70℃, the reaction time of 5.4 h, and pH of 9.2. Under this condition, the content of phosphate of Ph-FVSP was 7.53±0.13%. These results suggested that the established model was proved to be useful for the phosphorylation of the Ph-FVSP process.

Characterization of FVSP and Ph-FVSP

The FT-IR spectra of FVSP and Ph-FVSP was shown in Fig. 3. The infrared absorption spectra of the two samples was relatively similar, with strong absorption peaks near the 3400 cm^{-1}, which were attributed to the stretching vibration of the hydroxyl group in polysaccharide molecules (Sun *et al.*, 2018). The absorption bands around 1619 and 1616 cm^{-1} belonged to the COO-stretching vibration of uronic acids. The absorption peaks around 1386 and 1382 cm^{-1} belonged to the stretching vibration of C-OH and the O-CO-symmetric stretching vibration of the carboxylate group (Ming *et al.*, 2017). The absorption bands around 1079 and 1087 cm^{-1} were contributed by the vibrations of pyranoid rings (Cheng *et al.*, 2016) There were new peaks of Ph-FVSP at around 1250 and 894 cm^{-1}, which corresponded to characteristic absorptions of P = O and C-O-P, respectively (Wang *et al.*, 2018ab). The results suggested that hydroxyl groups in Ph-FVSP were phosphorylated successfully.

Antioxidant Activities of FVSP and Ph-FVSP

Polysaccharides could scavenge free radicals and inhibit lipid peroxidation due to their structural characteristics. The antioxidant activity of FVSP and Ph-FVSP was shown in Fig. 4 (A-C). As shown in Fig. 4(A), compared with FVSP, Ph-FVSP exhibited a better hydroxyl radical-scavenging effect. When the concentration was 1.0 mg/mL, the scavenging activity of Ph-FVSP against hydroxyl radicals was 45.2±0.78%, which was 80% higher than that of FVSP. This result was consistent with the result that the phosphorylated polysaccharide from pumpkin and ginseng showed higher antioxidant activities than native polysaccharides (Chen and Huang, 2019a; Xiong *et al.*, 2019). The increase in hydroxyl radical scavenging ability may be due to changes in the molecular structure of phosphorylated polysaccharides (Xia *et al.*, 2021).

Figure 4(B) indicated that both FVSP and Ph-FVSP had a poor ability to scavenge DPPH radicals within the concentration range of 0-1.0 mg/mL and the scavenging activity of Ph-FVSP was lower than that of FVSP. The scavenging activity of FVSP and Ph-FVSP against DPPH free radicals were 16.9±0.26 and 14.5±0.18%, respectively, which might be due to the change of structure.

The scavenging activity of FVSP and Ph-FVSP against superoxide anion radicals was shown in Fig. 4(C). The scavenging abilities of FVSP and Ph-FVSP against superoxide anion radicals were in a concentration-dependent manner. Compared with FVSP, the scavenging activity of Ph-FVSP against superoxide anion radicals improved significantly. The scavenging activities of Ph-FVSP reached 89.21±1.78% at 1.0 mg/mL. These results were consistent with the reports about phosphorylated polysaccharides from *Enteromorpha* linza (Wang *et al.*, 2013) and phosphorylated derivatives of garlic polysaccharides (Chen and Huang, 2019b).

Table 2: Experimental design and results for response surface analysis

Runs	*A*	*B*	*C*	*D*	Phosphate content (%)
1	-1	-1	0	0	6.34
2	0	0	1	1	7.23
3	0	0	0	0	7.52
4	-1	0	0	1	6.43
5	0	1	0	-1	6.56
6	0	1	-1	0	6.47
7	1	0	1	0	7.15
8	0	1	1	0	6.86
9	1	-1	0	0	6.53
10	-1	0	1	0	6.77
11	1	1	0	0	6.59
12	0	0	-1	1	6.99
13	0	1	0	1	6.65
14	0	-1	0	-1	6.09
15	1	0	-1	0	6.57
16	-1	0	-1	0	6.51
17	-1	0	0	-1	6.78
18	1	0	0	1	6.87
19	0	-1	-1	0	6.53
20	0	0	0	0	7.45
21	0	0	0	0	7.67
22	1	0	0	-1	6.31
23	0	0	1	-1	7.12
24	0	-1	1	0	6.85
25	0	0	-1	-1	6.54
26	0	0	0	0	7.39
27	0	0	0	0	7.64
28	-1	1	0	0	6.31
29	0	-1	0	1	6.90

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

Source of variation	Sum of squares	df	Mean square	F-value	p-value
Model	4.939839	14	0.352846	25.988440	<0.0001**
A	0.064533	1	0.064533	4.753128	0.0468*
B	0.003333	1	0.003333	0.245513	0.6279
C	0.468075	1	0.468075	34.475520	<0.0001**
D	0.232408	1	0.232408	17.117770	0.0010**
AB	0.002025	1	0.002025	0.149149	0.7052
AC	0.025600	1	0.025600	1.885538	0.1913
AD	0.207025	1	0.207025	15.248190	0.0016**
BC	0.001225	1	0.001225	0.090226	0.7683
BD	0.129600	1	0.129600	9.545538	0.0080**
CD	0.028900	1	0.028900	2.128596	0.1666
A^2	1.867600	1	1.867600	137.555900	<0.0001**
B^2	2.308616	1	2.308616	170.038500	<0.0001**
C^2	0.351641	1	0.351641	25.899730	0.0002**
D^2	0.901652	1	0.901652	66.410140	<0.0001**
Residual	0.190078	14	0.013577		
Lake of fit	0.132358	10	0.013236	0.917244	0.5877
Pure error	0.057720	4	0.014430		
Total	5.129917	28			
R^2	0.962900				
R_{adj}^2	0.925900				
C.V.%	1.710000				

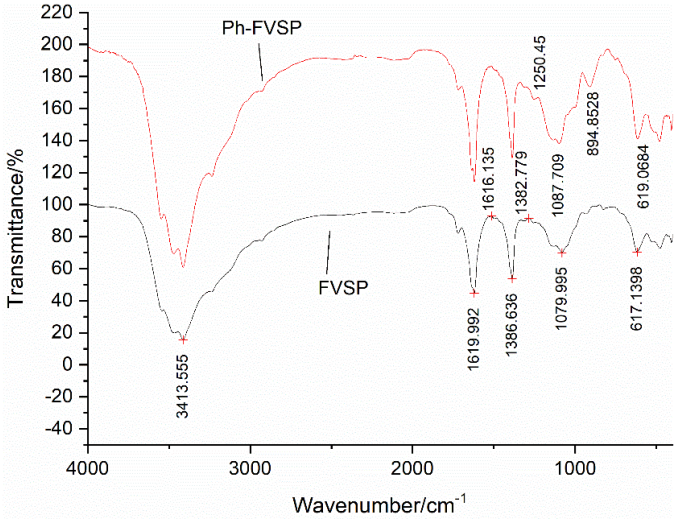


Fig. 3: FT-IR spectra of FVSP and Ph-FVSP

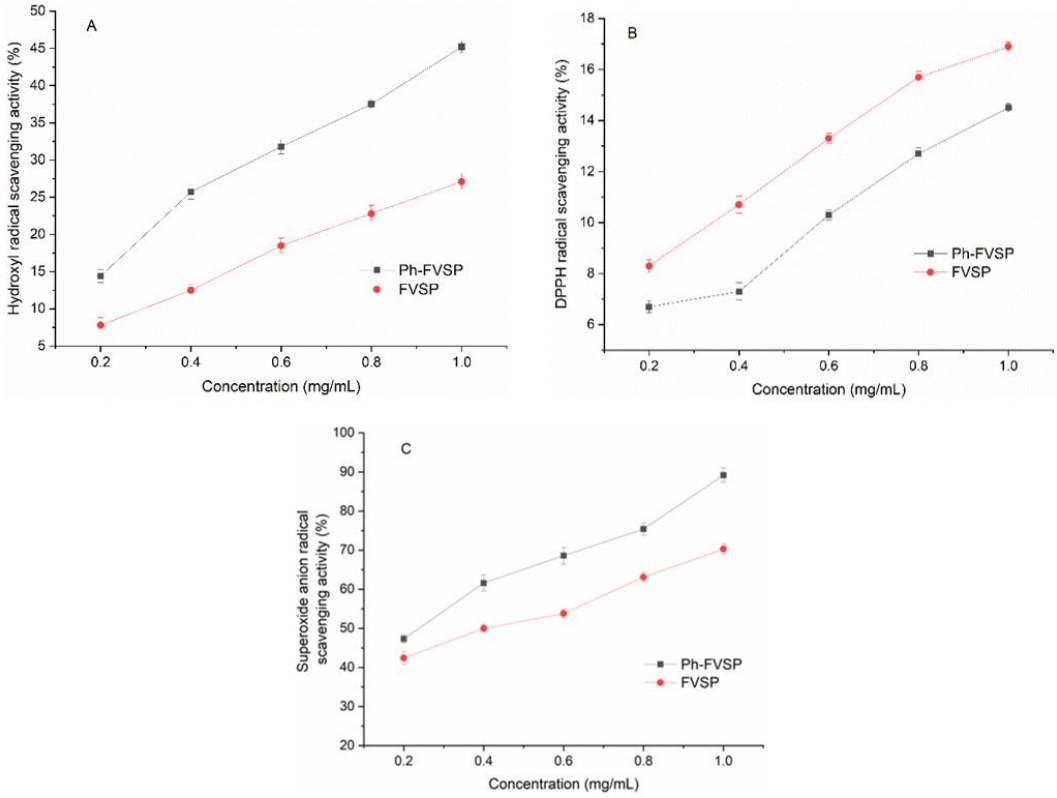


Fig. 4: The scavenging activities of FVSP and Ph-FVSP on hydroxyl radicals(A), DPPH radicals(B), and superoxide anion radicals(C)

Conclusion

In this study, FVSP was extracted from *F. velutipes* scraps and was phosphorylated to synthesize Ph-FVSP. The optimum preparation conditions of Ph-FVSP were obtained. Under the optimum conditions, the content of phosphate of Ph-FVSP was 7.53±0.13%.

Infrared spectroscopy suggested that the phosphorylation of FVSP was successful. Compared with FVSP, Ph-FVSP was more capable of scavenging hydroxyl radicals and superoxide anions. The results revealed that Ph-FVSP had potential as a novel food additive and antioxidant in the food industry. This study provided a method for FVSP phosphorylation. In the

future, more biological activities of Ph-FVSP such as anti-tumor activity, anti-viral activity, and immunomodulatory activity of Ph-FVSP will be further studied.

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Author's Contributions

Yingyun Peng and Xue Bai: Research in all experiments and article writing.

Hanwen Xu, Yuan Jin and Yufeng Wu: Research on characterization and antioxidant activity of Ph-FVSP and data processing.

Yiyong Chen: Project design, experimental guidance, and article writing of this 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.

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