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Research Article
Optimized Extraction of Soluble Dietary Fiber from *Lentinus edodes* by using Response Surface Methodology

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Abstract
Background and Objective: Stalk of *Lentinus edodes*, which often discarded in large amounts after *Lentinus edodes* process is a kind of material that is rich in dietary fiber. The objective of study was to obtain a suitable way to extract Soluble Dietary Fiber (SDF) from the stalks of *Lentinus edodes* and provide theoretical basis for further processing of it. **Methodology:** According to single-factor and response surface methodologies, suitable conditions for extracting SDF were determined through enzymatic hydrolysis based on extraction yield of water-SDF and one-way-analysis of variance (ANOVA) followed by Least Significant Difference test (LSD) and Duncan's multiple range test (Duncan) at $p<0.05$ were used to verify significant differences in evaluated parameters. **Results:** The optimal conditions for the SDF extraction rate are 52°C extraction temperature, 87 U g^{-1} enzyme concentration, 100 mesh number and 50 min extraction duration. On this condition, the SDF extraction rate is 14.33%. Water holding capacity and swelling property of SDF from stalks of *Lentinus edodes* measured 10 g/g and 0.93 mL g^{-1}, respectively. **Conclusion:** It is concluded that stalks of *Lentinus edodes* serve as good sources of SDF. Reliable and feasible optimization of SDF extraction from stalks of *Lentinus edodes* can be achieved through response surface method.

Key words: *Lentinus edodes*, dietary fiber, enzymatic hydrolysis method, response surface analysis, functional properties

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Based on repeated discussions conducted in December 1999, the American Association of Cereal Chemists defined Dietary Fiber (DF) as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation[1]." Based on the description and a large number of research, DF plays an indispensable role in reducing incidence rate of cancer and some chronic diseases, maintaining intestinal health, lowering cholesterol level and regulating blood sugar levels in the body[2]. The DF is generally classified as Soluble DF (SDF) and Insoluble DF (IDF)[3]. The DF features strong Water Holding Capacity (WHC) due to numerous hydrophilic groups in its structure[4]. The SDF shows stronger WHC than IDF[5]. With regard to physiological activity, SDF performs better than IDF in terms of application, sensory, physical activity and antioxidant activity[6].

Lentinus edodes ((Berk.) Sing.) is a fungus belonging to Omphalocele, Agaricales and Agaricomycetes and is a popular food choice in commercial markets due to its peculiar flavor and abundant nutritional value[7]. *Lentinus edodes* is the second most produced and consumed edible mushroom worldwide; in China, total yield of commercial *Lentinus edodes* reached 22 million tons in 2010[8,9]. Thus, million tons of waste mushroom rods, offals and mushrooms with defect were also produced. With increasingly popular large-scale agro-industrial production, dealing with waste materials and upgrading low-value byproducts, such as through deep processing technology and DF extraction, can contribute to recycling waste, generating revenues, utilizing resources and protecting the environment[10-12]. Preparation methods of DF mainly include chemical extraction, enzymatic hydrolysis extraction, physical extraction, membrane separation and fermentation. Liu *et al.*[13] used chemical extract method (alkaline process) on stalks of *Lentinus edodes* to extract SDF. Chemical extract method is simple and smart. However, color and purity of product is generally poor, use of alkaline easily corrupts the instrument and improper handling easily causes pollution to the environment. Final extraction rate is also undesirable. Enzymatic hydrolysis is a mild, efficient, energy-saving and environment-friendly method with significant potential to enhance yield of target products[7]. Extraction rate of SDF is related to particle size of materials[14]. However, influence of particle size is usually disregarded during experimentation. Therefore, extraction rate of SDF is relatively low.

Based on innovation points from the previous discussion, technological conditions of extracting SDF from stalks of *Lentinus edodes* are optimized by response surface analysis. This study aimed to provide theoretical data for comprehensive development and utilization of new SDF.

MATERIALS AND METHODS

Materials and Reagents: Stalks of *Lentinus edodes* were purchased in April, 2016, from a farmhouse in Jingmen, Hubei province, China. Samples were dried in thermostatic drum-wind drying oven (DHG-914385-lll, Shanghai) until moisture content was reduced to 7%. Stalks of *Lentinus edodes* feature the following composition: ~15.380% (g/100 g sample) protein, ~7% moisture, ~1.82% fat, ~61.286% DF (~6.084% soluble fiber) and ~6.55% ashes. Using a sieve, dried samples were homogeneously powdered to ensure that particle size meets requirements. Samples were then placed in a dry location. Cellulase (10000 U g^{-1}) was obtained from Nuoao (Tianjin, China). Anhydrous ethanol, sodium citrate and citric acid were all of analytical grade.

Extraction procedures: Enzyme powder was dissolved in citric acid and sodium citrate buffer solution (0.05 mol L^{-1}, pH 5.0, preparing when using) and enzymolysis solution was placed in refrigerator at 4°C until use. Under the defined condition of single factor, 1.0000 g of pre treated sample powder was complementarily dispersed in 20 mL buffer solution (0.05 mol L^{-1}, pH 5.0) by using a magnetic stirring apparatus (79-2, Jintan Jieruier Electric Appliance Co., Ltd, Jiangsun, China). Enzymolysis solution for enzymatic hydrolysis was placed in water-bath box at constant temperature. During enzymatic hydrolysis, the mixed solution was stirred every 10 min. Enzyme activity was terminated by placing the solution in 100°C water bath for 10 min. The solution was rapidly cooled to room temperature. Extract solution was centrifuged (6000 rpm for 5 min at 17°C) to obtain the supernatant, which was placed in a round-bottomed flask. Supernatant was concentrated to one quarter of its original volume using rotary evaporator under vacuum. Concentrated extract was then precipitated by adding four volumes of anhydrous ethanol and storing resulting mixture overnight at room temperature. After suction filtration, collected precipitates were dried in drum-wind drying oven at 50°C. The SDF extraction rate (%) represents the ratio of dried crude SDF weight (g) and sample weight (g).

Determination of WHC: The WHC was determined according to Huang and Ma[15], who defined environmental temperature as 37°C and modified centrifugal condition to 3000 rpm for 15 min.

Determination of swelling property: Swelling property was determined according to Huang and Ma[15], who defined environmental temperature as 37°C.

Statistical analysis: All experiments were performed in triplicate and results were described as Mean value±Standard Deviation (SD) (n = 3) in the part of the single-factor test and function properties results analysis. All data were analyzed by SPSS 17.0 and Design-Expert.8.05b, respectively. One-way-analysis of variance (ANOVA) followed by Least Significant Difference test (LSD) and Duncan's multiple range test (Duncan) at $p<0.05$ were used to verify significant differences in evaluated parameters[16].

RESULTS AND DISCUSSION

The SDF performs well in reducing risk of cancer and some chronic diseases, maintaining intestinal health and lower cholesterol and regulating blood sugar levels in the body. Optimizing suitable conditions of extracting SDF from stalks of *Lentinus edodes* can contribute to developing new sources of DF, fully utilizing edible fungi resources to improve human health and promoting development of large-scale *Lentinus edodes* production in the future. Using stalks of *Lentinus edodes* as raw material can serve as concrete reflection of saving resources and protecting the environment. Results of this study offer an economical, environmentally friendly and practical way of recycling cheap byproducts into products with health benefits.

Effects of extraction temperature on extraction rate of SDF: The SDF extraction is an enzymatic hydrolysis reaction that is influenced by various factors. As shown in Fig. 1a, extraction temperature can significantly ($p<0.05$) affect extraction. Extraction temperature showed a parabolic curve relationship with extraction rate. The highest extraction rate of 12.51% was achieved at 51°C. This phenomenon occurred because SDF primarily comprises natural pectin and β-glucan. High temperature damaged structure of compounds and thus reduced extraction rate of SDF[13]. On the contrary, when extraction temperature was excessively higher than the suitable temperature for the enzyme, enzyme activity was significantly affected ($p<0.05$), thereby possibly influencing enzyme performance[17,18]. Therefore, appropriate extraction temperature was set at 51°C.

Effects of enzyme concentration on extraction rate of SDF: Enzyme concentration plays an important role in enzymatic hydrolysis reaction. Inspection points were set at 10, 50, 90, 130, 170 and 210 U g^{-1} to determine economical and reasonable enzyme concentration. Results (Fig. 1b) showed that extraction rate initially increased and then decreased after peaking at 90 U g^{-1} and this trend was considered significant ($p<0.05$). Possibly and as described in the instrument, the enzyme cannot fully function when it is only present in small amounts. Thus, enzyme concentration played a decisive role in this condition and extraction rate increased with increasing enzyme concentration. However, when addition went beyond the limit, SDF was broken down and generated monosaccharides, oligosaccharides and other small molecules that cannot be precipitated in anhydrous ethanol, leading to low extraction rate. Therefore, cellulase content would measure 90 U g^{-1} to obtain high extraction yield and controls low costs.

Effects of mesh number on extraction rate of SDF: Research on SDF extraction often disregarded effects of mesh number. Figure 1c shows that mesh number significantly ($p<0.05$) affected extraction rate of SDF. The SDF extraction rate for the sample with particle diameter of 180 μm was high with aperture of 80-mesh sieve compared with extraction from the sample with significantly small or significantly large particle diameter. Increases in specific surface area, porosity, solubility, dispersion and adsorption of sample due to crushing-disposal were advantageous to dissolution and extraction of SDF from material internal[19] and enhancement of affinity between cellulose and enzyme[20]. Extensive bonding and cross-linking of lignin and hemicellulose with cellulose limit accessibility of enzymes to degrade polysaccharides and release fermentable monosaccharides[21] during pulverization and under actions of shear force and extrusion force. Insoluble high polymers are prone to fracture, thereby increasing extraction content of small-molecular SDF. Nonetheless, excessive crushing results in degradation by cellulase of some low molecular weight substances to simple sugars, oligosaccharides and other small molecules which cannot be obtained through alcohol precipitation. Consequently, 80 meshes are appropriate under single-factor conditions.

Effects of extraction duration on extraction rate of SDF: Extraction duration is another factor influencing extraction of

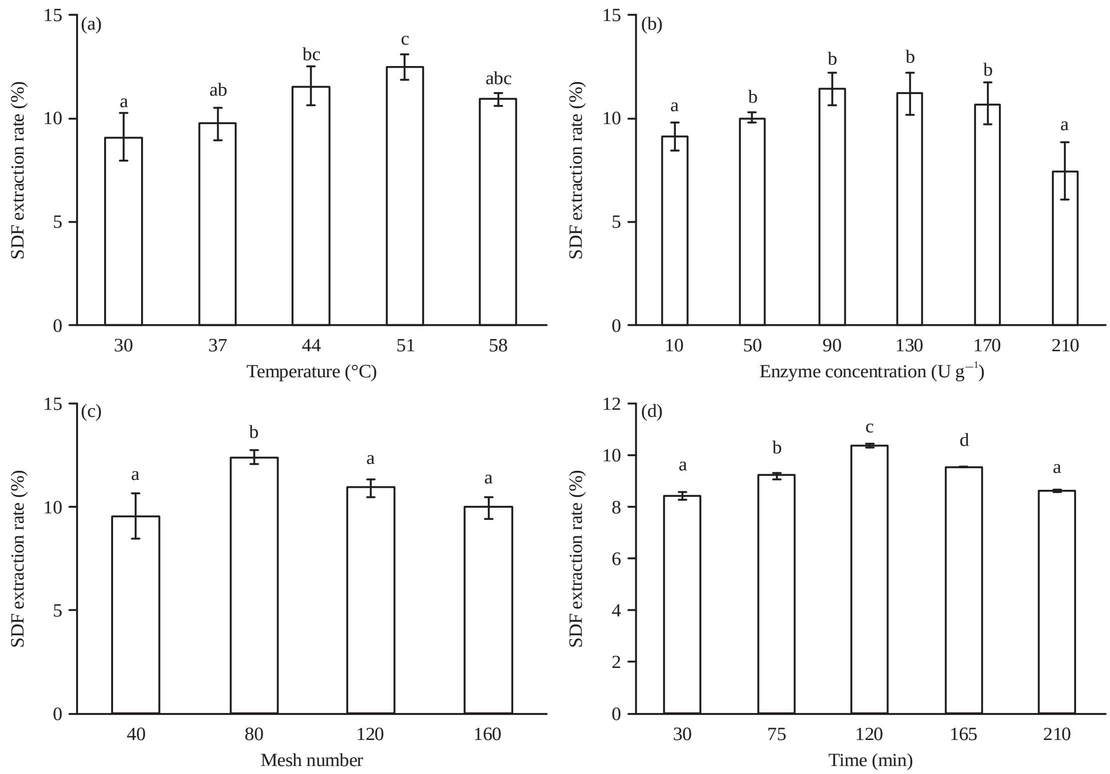

Fig.1(a-d): Effects of (a) Extraction temperature, (b) Enzyme concentration, (c) Mesh number and (d) Extraction duration on extraction rate of SDF

The error bars mean the size of standard deviation(SD)

SDF. As shown in Fig. 1d, extraction duration significantly ($p<0.05$) affected extraction and showed a parabolic curve relationship with extraction rate. After 120 min of extraction, extraction yield peaked. On the one hand, adequate time can aid cellulose in hydrolyzing structure-like hemicellulose-heteroxylan into smaller molecules or segments[22]. On the other hand, owing to poor solubility of protopectin of pectin, which is the major component of SDF, short extraction did not fully dissolve protopectin. Hence, appropriate extension of enzyme action time can aid dissolution of pectic substances and thus improve extraction rate of SDF. According to enzymatic kinetics of cellulose degradation, long-time enzyme action reduced enzyme concentration, inactivated a part of enzymes, enhanced product feedback inhibition effect and reduced velocity of enzyme-promoting reaction. The amount of pectin, which is degraded by lipase and hydrolyzed by hydrogen ions, increased and extraction of SDF reduced. Thus, 120 min was considered the appropriate time for SDF extraction.

Based on single-factor experiments, a quadratic regression equation was designed to fit the functional relationship between independent variables and extraction yield according to the principle of Box-Behnken center combination experimental design. Extraction temperature, enzyme concentration, mesh number and extraction duration served as independent variables and extraction rate of SDF was the response value. Table 1 presents range and center point values of four independent variables.

Twenty-nine groups of experiment were conducted according to the established design. Table 2 shows experimental design scheme and results. Conditions of experimental factors of 2, 10, 16, 25 and 29 run were maintained consistent (all experiment factors were at 0 level) to verify pure error of experiments. Data were analyzed through multiple regressions using Design-Expert 8.05b. A quadratic multinomial regression model was established to determine the relationship of SDF extraction yield (Y) and independent variables, namely, extraction temperature (A), enzyme concentration (B), mesh number (C) and extraction duration (D). The quadratic regression equation is calculated as follows:

$$Y = 14.20+0.20\times A+0.069\times B-0.45\times C-0.72\times D+0.30\times A\times B-0.57\times A\times C+0.14\times A\times D+0.67\times B\times C-0.14\times B\times D+0.85\times C\times D-1.49\times A^2-0.47\times B^2-1.63\times C^2-0.87\times D^2$$

Analysis of variation using p and f-values: Statistical significance of regression equation was determined by F-test and ANOVA and results are presented in Table 3. Model F-value of 8.74 and values of "Prob>F" (0.0001) indicated that the model was extremely significant. In this case, D, A^2, C^2 and D^2 are extremely significant model terms, whereas C, BC and CD are significant model terms. The "Lack of Fit F-value" of 2.73 and p = 0.1731 imply that Lack of Fit was not significant relative to pure error. A 17.31% chance indicates that such high "Lack of Fit F-value" can occur due to noise. Non-significant lack of fit was good. Score of variation (CV) reached 1.44% and the model can explain 89.73% chance in response. "Adeq Precision" measures signal-to-noise ratio and is shown in Table 4. A ratio >4 was considered desirable. Hence, "Adeq Precision" of 9.205 indicates an adequate signal. In conclusion, this model can be used to navigate the design space.

Using Design-Expert. 8.05 b, response surface results were summarized to explore interactive effects of temperature, enzyme concentration, mesh number and extraction duration on SDF extraction rate. Response surface plot and contour plot were obtained for the yield of SDF as a function of experimental conditions. As shown in Fig. 2, each 3D graph exhibited a parabolic surface. The highest SDF extraction rate

Table 1: Factors and levels in four-factor and three-level response surface analysis

	Coded levels		
Variables	-1	0	1
Temperature (°C)	37	51	65
Enzyme concentration ($U g^{-1}$)	10	90	170
Mesh number	80	120	160
Extraction duration (min)	30	75	120

Table 2: Response surface central composition design arrangement and experimental results

Test number	A	B	C	D	Y (%)
1	0	0	-1	-1	13.38
2	0	0	0	0	14.08
3	-1	0	0	-1	13.06
4	-1	0	1	0	11.23
5	1	1	0	0	12.70
6	0	-1	-1	0	14.01
7	1	-1	0	0	12.17
8	-1	0	-1	0	10.48
9	1	0	0	-1	12.34
10	0	0	0	0	14.18
11	0	1	0	1	12.70
12	-1	-1	0	0	11.86
13	0	-1	0	1	11.82
14	0	-1	0	-1	13.03
15	0	1	1	0	11.82
16	0	0	0	0	13.76
17	0	-1	1	0	11.14
18	-1	0	0	1	11.33
19	-1	1	0	0	11.19
20	0	1	-1	0	11.99
21	0	0	1	1	11.19
22	0	0	1	-1	10.90
23	1	0	1	0	10.82
24	1	0	-1	0	12.37
25	0	0	0	0	14.14
26	1	0	0	1	11.16
27	0	1	0	-1	14.46
28	0	0	-1	1	10.29
29	0	0	0	0	14.85

A: Temperature, B: Enzyme concentration, C: Mesh number, D: Extraction duration, Y: Extraction rate of SDF

Table 3: Analysis of variance of the factors for SDF extraction yield

Sources	Sum of square	df	Mean square	F-value	Prob>F	Significance
Model	43.32	14	3.09	8.74	0.0001	**
A	0.48	1	0.48	1.37	0.2619	
B	0.057	1	0.057	0.16	0.6933	
C	2.45	1	2.45	6.91	0.0198	
D	6.28	1	6.28	17.73	0.0009	
AB	0.36	1	0.36	1.02	0.3305	
AC	1.32	1	1.32	3.73	0.0738	
AD	0.076	1	0.076	0.21	0.6511	
BC	1.82	1	1.82	5.15	0.0396	
BD	0.076	1	0.076	0.21	0.6511	
CD	2.86	1	2.86	8.06	0.0131	
A^2	14.38	1	14.38	40.60	<0.0001	
B^2	1.41	1	1.41	3.98	0.0658	
C^2	17.13	1	17.13	48.37	<0.0001	
D^2	4.91	1	4.91	13.87	0.0023	
Residual	4.96	14	0.35			
Lack of fit	4.32	10	0.43	2.73	0.1731	NS
Pure error	0.63	4	0.16			
Cor total	48.28	28				

**Extreme significance (p<0.01), *Significance (p<0.05). df: Degrees of freedom, NS: Non significance, SDF: Soluble dietary fiber

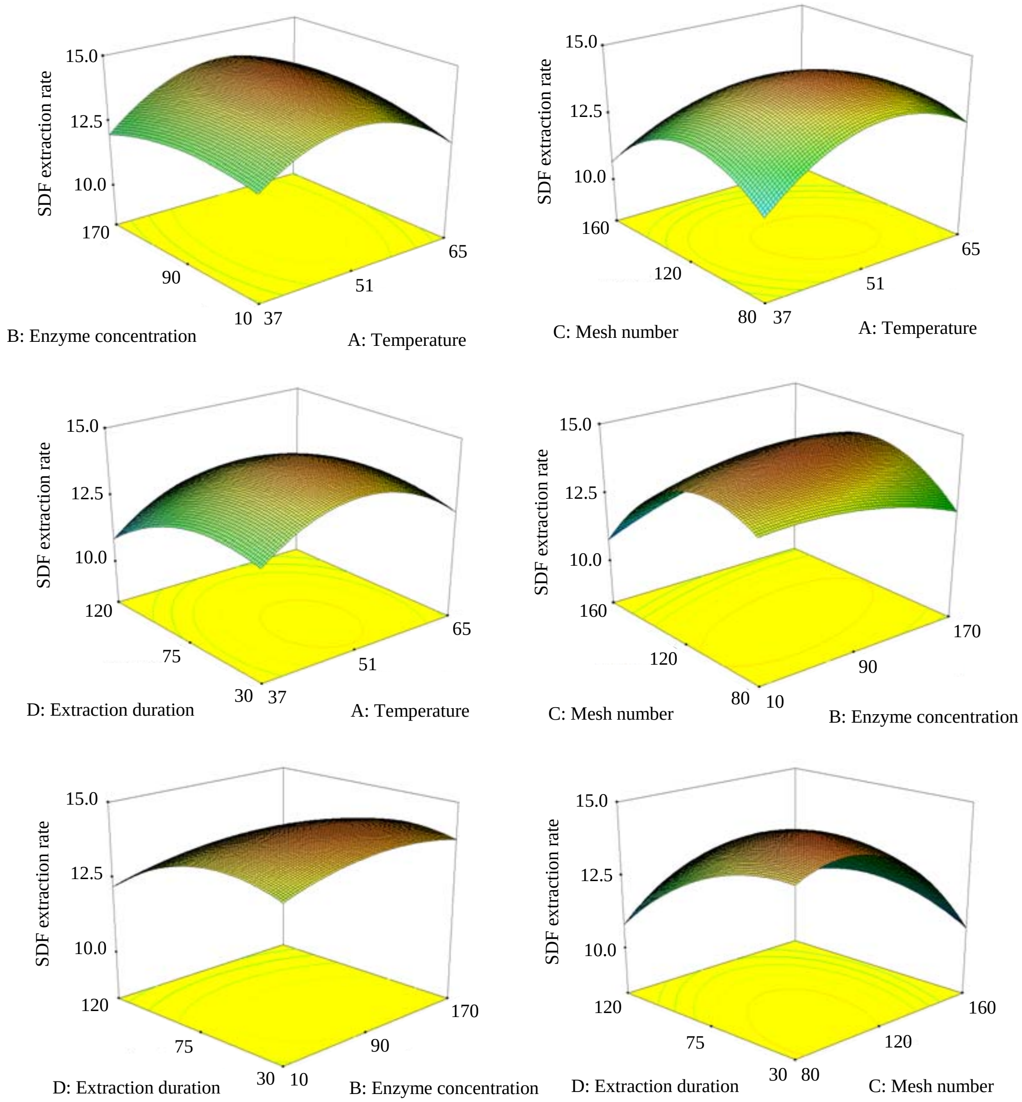

Fig. 2: Response surface diagrams of the four varied parameters and SDF extraction yields

was achieved during interaction of two factors. The corresponding contour plot also displayed a clear peak. According to the quadratic polynomial regression model and response surface analysis results, the regression model, which was calculated, confirms the stable point. According to analysis of results of response surface test by Design-Expert.8.05b, suitable SDF extraction conditions were as follows: extraction temperature of 52.38°C, enzyme concentration of 87.20 U g^{-1}, mesh number equals 107.67 and extraction duration totaling 50.03 min. Under these conditions, predicted maximum extraction rate totaled 14.4806%. Considering operability of reaction, suitable parameters included extraction temperature of 52°C, enzyme concentration of 87 U g^{-1}, mesh number of 100 and extraction duration of 50 min. Under these conditions, SDF extraction rate reached 14.33%, which agrees with the predicted value.

Functional properties: As shown in Table 5, significant differences ($p<0.05$) in WHC and swelling property were detected between *Lentinus edodes* stem and extracted SDF. The SDF showed significantly better WHC ($p<0.05$) than *Lentinus edodes* stem raw material. However, the raw material exhibited significantly better swelling property ($p<0.05$) than SDF. Compared with bran DF (WHC, 4.0 g/g, swelling capacity, 4.0 mL g^{-1}) (analysis of DF in food), which is popular in Western countries, SDF extracted from *Lentinus edodes* stem features remarkable advantages in WHC.

Table 4: Credibility analysis of regression equation

Items	Values	Items	Values
Standard deviation	0.60	R-Squared	0.8973
Mean	12.36	Adj R-Squared	0.7946
CV (%)	4.81	Pred R-Squared	0.4636
PRESS	25.90	Adeq Precision	9.205

Table 5: Functional properties of SDF and *Lentinus edodes*

Samples	WHC (g/g)	Swelling property (mL g^{-1})
Lentinus edodes stem	6.60±0.063[a]	3.635±0.005[a]
SDF	10±0.009[b]	0.93±0.01[b]

SDF: Soluble dietary fiber, WHC: Water holding capacity, results are described as Mean value±Standard Deviation (SD) (n = 3), different letters within the same column indicate significant difference (p<0.05)

CONCLUSION

The following conclusions are drawn from this study:

- SDF was obtained from stalks of *Lentinus edodes*
- Four independent variables (temperature, enzyme concentration, mesh number and extraction duration) significantly affect extraction rate of SDF
- Suitable conditions of SDF extraction from stalks of *Lentinus edodes* were optimized
- Obtaining high quality of SDF from waste materials of *Lentinus edodes* and dealing and upgrading low-value byproducts which can contribute to recycling wastes, generating revenues, fully utilizing resources and protecting the environment
- Compared with bran DF (WHC, 4.0 g/g, swelling capacity, 4.0 mL g^{-1}), which is popular in Western countries, SDF extracted from *Lentinus edodes* stem shows significant advantages in WHC

SIGNIFICANCE STATEMENT

In this study, first time the suitable conditions of extracting SDF from the stalk of *Lentinus edodes* were optimized and the functional properties of the SDF were determined. Consequently, obtained a kind of reliable and feasible route to obtain a high quality SDF, meanwhile, taking the mushroom processing waste as a raw material was helpful to make full use of resources and protect the environment.

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