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Physicochemical properties, antioxidant activities and hypoglycemic effects of soluble dietary fibers purified from *Lentinula edodes*

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Abstract

Lentinula edodes (*L. edodes*), which imparts various health benefits to humans, is considered a novel source of soluble dietary fiber (SDF). In this study, ultrasonic-assisted hot-water method was used to extract SDF (U-SDF) from *L. edodes*, and physicochemical, antioxidant and hypoglycemic properties of the U-SDF were investigated. Physicochemical properties of U-SDF showed that water solubility, water holding capacity, swelling capacity, and oil holding capacity were higher than that the SDF extracted using hot water method without ultrasonication. The DPPH, •OH, and $\bullet O_2^-$ radical clearance rates indicated that U-SDF exhibited better antioxidant activities. U-SDF also exhibited notable α-amylase and α-glucosidase inhibition activities. Treatment with U-SDF alleviated glucose and peroxidation metabolism disorders *in vivo*. Histological analysis indicated that U-SDF improved the oxidative tissue damage in diabetic mice. These results provided a theoretical basis for the development and utilization of SDF derived from *L. edodes*.

Keywords: *Lentinula edodes*; soluble dietary fibers; physicochemical properties; antioxidant activities; hypoglycemic effects.

Practical Application: The U-SDF of *L. edodes* is an excellent source of dietary fiber, with better physicochemical properties, antioxidant activities and hypoglycemic effects. It has great potential for use as a functional additive in diverse food products in future.

1 Introduction

With the increasing concern about food and health, natural compounds have attracted significant interest owing to its high efficacy and low adverse effects in type 2 diabetes mellitus (T2DM) treatment (Altındağ et al., 2021). Dietary fibers (DFs) are a type of carbohydrate that cannot be hydrolyzed by endogenous enzymes in the small intestine of humans, but can be partially fermented and utilized by certain microorganisms in the large intestine (Mariah et al., 2021; Wu et al., 2020). DFs are divided into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) according to their solubility in water (Dhingra et al., 2012). SDF is associated with important physiological functions, which play a role in improving intestinal flora, preventing gastrointestinal diseases, regulating postprandial blood sugar, and preventing chronic diseases, including cardiovascular and cerebrovascular diseases (Huang et al., 2015).

Lentinula edodes (*L. edodes*) has become the second most popular edible and medicinal fungus available in the global market because of its higher nutritional value, better medicinal attributes, and rapid yielding capacity (Huang et al., 2019). *L. edodes* is reportedly rich in several bioactive compounds, such as carbohydrates, proteins, dietary fiber, lipids, vitamins, folates, niacin, and minerals, which have been isolated and identified from fruiting bodies, mycelia, and culture media, making it a high-protein and low-fat food (García-Segovia et al., 2011). *L. edodes*, which is associated with low calorie lipids as well as rich fiber composition, is considered a novel source of SDF, which imparts various health benefits to humans (Yuan et al., 2018). However, studies on the physicochemical properties, antioxidant activities and hypoglycemic effects of the SDF in *L. edodes* are limited.

In this study, we used an ultrasonic-assisted hot water extraction method to extract SDF from *L. edodes*. Physiochemical properties, antioxidant functions and hypoglycemic properties of U-SDF were also estimated. Thus, the current study aimed to provide a theoretical basis for the development and utilization of SDF derived from *L. edodes*.

2 Materials and methods

2.1 Materials

Air-dried *L. edodes,* purchased from a market in Xuzhou, China, were washed, dried, and cut into small pieces. The pieces were then pulverized and powdered using an XA-I high-speed pulverizer (JiangSu Dongpeng Instrument Manufacturing Co., Ltd.) and passed through a 60 mesh screen. Subsequently, the powder was soaked in 95% ethanol for 48h to remove fat-soluble substances. The soaked powder was then dried in an oven at 40 °C. The samples were placed in a dry container for further analysis.

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2.2 Extraction and preparation of U-SDF from L. edodes

Ultrasonic-assisted hot-water method was used to prepare the U-SDF. Briefly, *L. edodes* powder was dissolved in distilled water according to a certain liquid-solid ratio and ultrasonic treatment. Then, the temperature of a water bath was adjusted, and the solution was incubated in hot water for a certain time. Following suction filtration, the filtrate was collected and trichloroacetic acid was added to remove protein impurities. The filtrate was then centrifuged for 10 min at 8000 g. The filtrate was concentrated under reduced pressure in a rotary evaporator at 60 °C. Next, 95% ethanol was added and incubated at 25°C for 24h. The precipitate was collected via centrifugation at 8000 g for 10 min and washed twice with 100% ethanol. U-SDF was obtained after the precipitate was freeze-dried to a constant weight. The extraction yields were calculated using the following Equation 1:

$$\mathrm{Y}(\%) = \frac{W}{W_p} \times 100\% \quad (1)$$

where W is the weight of the U-SDF and W_p is the weight of the *L. edodes* powder.

2.3 Water solubility (WS) analysis

In brief, a 1.0 g U-SDF sample (W_1) was mixed with 50 mL distilled water and stirred until the mixture was evenly dispersed and then centrifuged at 5000 g for 12 min. The supernatant was collected, dried to a constant weight at 105 °C in an oven, and weighed as W_2. WS was determined using the following Formula 2:

$$\mathrm{WS\ (ml/g)} = \frac{W_2}{W_1} \times 100\% \quad (2)$$

2.4 Water holding capacity (WHC) analysis

Briefly, 1.0 g of the U-SDF sample (W_1) was mixed with 30 mL of distilled water. The mixture was incubated for 12h at 25 °C and filtered through a filter paper. The filtered samples were collected and weighed (W_2). WHC was calculated as Formula 3:

$$\mathrm{WHC\ (g/g)} = \frac{W_2 - W_1}{W_1} \quad (3)$$

2.5 Oil holding capacity (OHC) analysis

In brief, a 1.0 g U-SDF sample (O_1) was added to 20 mL of soybean oil while mixing vigorously. The mixture was incubated for 12h at 25 °C, and then centrifuged at 4000 g for 15 min to remove the supernatant (free oil). The residue was collected and weighed using an O_2. The OHC was determined using the following Formula 4:

$$\mathrm{OHC\ (g/g)} = \frac{O_2 - O_1}{O_1} \quad (4)$$

2.6 Swelling capacity (SC) analysis

In brief, a 1.0 g U-SDF sample (W) was added to 15 mL of distilled water while mixing vigorously. The initial volume of the SDF samples was recorded as V_1. The mixed samples were incubated at 37 °C for 24h. The expansion volume of the samples was recorded as V_2, and SC was calculated as Formula 5:

$$\mathrm{SC\ (ml/g)} = \frac{V_2 - V_1}{W} \quad (5)$$

2.7 Determination of antioxidant activity in vitro

Trinitro-phenyl hydrazine (DPPH) radical scavenging activity

In brief, 2.0 mL of U-SDF (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) were uniformly mixed with 2.0 mL of DPPH-95% alcohol solutions (0.1 mmol/L). The mixture was incubated for 30 min at room temperature in the dark and then centrifuged at 4000 g for 15 min to collect the supernatant. Absorbance of the supernatant was measured at 517 nm in an ultraviolet spectrophotometer. Vitamin C (V_C) was used as a positive control. DPPH radical scavenging activity (%) was determined using the following Equation 6:

$$\text{DPPH radicals scavenging activity (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (6)$$

where A_0 is the absorbance of the DPPH solution without U-SDF, A_1 is the absorbance of the U-SDF mixed with the DPPH solution, and A_2 is the absorbance of the U-SDF without DPPH solution.

•OH radical scavenging ability

Briefly, 2.0 mL of U-SDF (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) were mixed with 2mL of $FeSO_4$ (8 mmol/L) and 2 mL of salicylic acid (8 mmol/L). Subsequently, 2 mL of H_2O_2 (8 mmol/L) was added to the mixture and then incubated at 37 °C for 30 min. Absorbance of the mixture was measured at a wavelength of 510 nm. Distilled water was used as the blank control and V_C was used as the positive control. The •OH radical-scavenging activity (%) was calculated using the following Equation 7:

$$\cdot\mathrm{OH}\ \textit{radicals scavenging activity}\ (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (7)$$

where A_0, absorbance of the blank control; A_1, absorbance of the U-SDF; and A_2, absorbance of the U-SDF without H_2O_2.

•$O2^-$ radical scavenging ability

First, 5.0 mL of Tris–HCl (50 mmol/L, pH 7.4) containing Na_2-EDTA (1 mmol/L) and pyrogallol (60 mmol/L) was preheated at 37 °C for 20 min. Then, 4.0 mL of U-SDF (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) were uniformly mixed with the solution and incubated at 37 °C for 5 min, and the absorbance was detected at 325 nm. Distilled water, which replaced U-SDF, was used as the blank control, and V_C was used as the positive control. The scavenging activity of $\cdot O_2^-$ radicals (%) was calculated using the following Equation 8:

$$\cdot\mathrm{O}_2^-\ \text{scavenging activity}\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100\% \quad (8)$$

where A_0 is the absorbance of the blank control and A_1 is the absorbance of the U-SDF.

2.8 Determination of hypoglycemic activity in vitro

α-Amylase activity inhibition

Briefly, 100 µL of α-amylase (5U/mL) (Sigma, USA) and 50 µL of U-SDF (0.5, 1, 1.5, 2, and 2.5 mg/mL) were mixed

and incubated at 25 °C for 10 min. Then 50 μL of 1% starch solution was added and incubated for 10 min. The reaction was terminated by adding 100 μL of 3, 5-dinitrosalicylic acid (10 mg/mL). The absorbance was measured at 540 nm after boiling water bath for 10 min. Acarbose was used as the positive control. The inhibition rate was calculated using the following Equation 9:

$$\alpha\text{-Amylase activity inhibition rate}(\%) = 1 - \frac{A_1 - A_2}{A_0} \times 100\% \quad (9)$$

where A_1 is the absorbance of U-SDF with the α-amylase, A_2 is the absorbance of U-SDF without the α-amylase, and A_0 is the absorbance of α-amylase without the U-SDF.

α-glucosidase activity inhibition

Briefly, 100 μL of α-glucosidase (0.35 U/mL) (Sigma, USA) and 50 μL of U-SDF (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) were combined for 20 min at 37 °C. Subsequently, 5 0 μL of p-Nitrophenyl α-D-glucopyranoside (PNPG) (1.5 mmol/L) was added to the mixture and further incubated at 37 °C for 20 min. The reaction was terminated by addition of 100 μL Na_2CO_3 (1 mol/L), and the absorption was detected at 405 nm. Acarbose was served as the positive control. Inhibition rates were calculated using the following Equation 10:

$$\alpha\text{-glucosidase activity inhibition}(\%) = 1 - \frac{A_1 - A_2}{A_0} \times 100\% \quad (10)$$

where A_1 corresponds to the sample absorbance value, A_2 corresponds to the absorbance value for the U-SDF without α-glucosidase, A_0 corresponds to the absorbance of α-glucosidase without U-SDF.

2.9 Induction of the diabetic mice model

Male C57/BL6J mice (20.0 ± 2.0 g) of SPF grade were used for this experiment and were purchased from Pengyue Experimental Animal Breeding Co., Ltd. (Jinan, China). The diabetic mouse model was established as described in our previous study (Ni et al., 2017). Briefly, the mice were randomly divided into control group (n = 5) and diabetic model group (n = 20) after one week of adaptive feeding. The mice of control were fed with the normal diet during the experiment. The mice of diabetic model group (n = 20) were fed with a high-fat diet for four weeks and then they were given a single intraperitoneal injection of STZ (100 mg/kg) after being fasted overnight to induce hyperglycemia. The mice of control were only injected with an equal volume citrate buffer. Fasting blood glucose levels were monitored after one week of STZ injection. The mice with fasting blood glucose levels higher than 11.1 mmol/L were considered to be diabetic. The diabetic mice were randomly divided into four groups randomly. They were list as below: (1) diabetic control group (DG, n = 5), treated with saline; (2) diabetic groups treated with U-SDF at doses of 250 mg/kg (U-S-250), 500 mg/kg (U-S-500), and 1000 mg/kg (U-S-1000) body weight, respectively. All animals were given U-SDF orally once daily for 3 weeks. Food intake, water intake and body weight were measured daily, and blood glucose levels were measured once a week throughout the study. Oral glucose tolerance test (OGTT) was performed as described in our previous study (Ni et al., 2016). The area under the curve (AUC) was calculated by the trapezoid method follow the formula: AUC (min ▪ mmol/L) = [1/2 × BG (0 min) + BG (120 min)] +[BG (90 min) + BG (60 min) - BG (30 min)] × 30 min. Homeostatic model assessment (HOMA) was used to assess the changes of insulin resistance (HOMA-IR), insulin sensitive (HOMA-IS). The Equations 11 and 12 are as follows:

$$\text{HOMA} - \text{IR} = \text{FBG*FINS} / 22.5 \quad (11)$$

$$\text{HOMA} - \text{IS} = 1 / \text{FBG*FINS} \quad (12)$$

The animal experimental protocols were approved by the Experimental Animal Ethics Committee of Xuzhou Medical University (approval number: L20210226457).

2.10 Biochemical analysis

Mice were sacrificed by cervical dislocation at the end of study and collected the blood from the orbital. The blood was stewing at 4 °C for 30 min and then centrifuged at 3000 g for 10 min to collect the serum. Dissecting the mice and collecting the pancreas and liver tissues. The MDA content and the activities of SOD in the serum and liver were determined by the commercial kits and normalized by protein content. All kits were used according to the manufacturer's recommendations. Small piece of tissues was cut and fixed in 10% neutral formalin solution, dehydrated in a graded series of ethanol, and embedded in paraffin for histopathological examination (HE).

2.11 Statistical analysis

Values are expressed as mean ± standard deviation (SD). Experimental data analyses were performed using GraphPad Prism version 8.0.2. Statistically significant differences were evaluated using ANOVA. Differences between samples were considered statistically significant at $p < 0.05$.

3 Results

3.1 Physicochemical properties of U-SDF from L. edodes

The solubility of DFs is mainly determined by the stability of their special ordered and disordered structure. DFs with irregular structures have better solubility. This study found that the WS of U-SDF was significantly higher than that the control without ultrasonic treatment (84.94 ± 2.30 vs. 91.41 ± 1.60, $p < 0.05$) (Table 1). Hydration properties, including WHC and SC, are important factors for evaluating the physiological functions of DFs. The WHC (2.03 ± 0.25 vs. 3.73 ± 0.10, $p < 0.01$) and SC (3.25 ± 0.25 vs. 5.22 ± 0.33, $p < 0.01$) of SDF extracted by ultrasonic assisted hot water extraction were improved compared with those of the control (Table 1). OHC is an important indicator, which indicates whether DFs have a good fat adsorption capacity. The OHC of U-SDF extracted by ultrasonic assisted hot water extraction was higher than that of the control (1.53 ± 0.27 vs. 3.45 ± 0.18, $p < 0.01$) (Table 1). This may be due to the surface area of the DFs being increased by ultrasonic modification, resulting in a relatively loose fiber structure, which enhanced oil penetration.

Table 1. Physical and chemical properties of U-SDF from *L. edodes*.

Sample	WS (%)	WHC (g/g)	SC (mL/g)	OHC (g/g)
Control [1]	84.94 ± 2.30	2.03 ± 0.25	3.25 ± 0.25	1.53 ± 0.27
U-SDF	91.41 ± 1.60 *	3.73 ± 0.10 **	5.22 ± 0.33 **	3.45 ± 0.18 **

[1]Control is the SDF extracted using hot water without ultrasonication. *$p < 0.05$, vs. control; **$p < 0.01$, vs. control.

3.2 Antioxidant properties of U-SDF from L. edodes

When the concentration ranged between 0.5 mg/mL to 2.5 mg/mL, the DPPH, •OH and $•O_2^-$ free radicals scavenging ability of U-SDF increased rapidly in a dose-dependent manner. At the same concentration, the DPPH radical scavenging ability of U-SDF was higher than the control but far below that of V_C. The DPPH free radical scavenging rate of U-SDF reached 80.84% ± 4.47% at a concentration of 2.5 mg/mL, while that of the control at the same concentration was 58.94% ± 5.46% (Figure 1a). When the concentration of the sample was 2.5 mg/mL, the •OH clearance rate of U-SDF reached 73.45% ± 2.66%, while that of the control reached 52.31% ± 4.63%. Although, the overall clearance of •OH by U-SDF was lower than that of V_C, it was better than that of the control at the same concentration (Figure 1b). The $•O_2^-$ radical clearance rate of U-SDF reached 73.63% ± 3.40% at a concentration of 1.5 mg/mL. Subsequently, as concentration continued to increase, the $•O_2^-$ radical scavenging rates of all groups gradually stabilized. The overall $•O_2^-$ radical clearance of U-SDF was lower than that of V_C but higher than that of the control at the same concentration (Figure 1c). These results indicated that U-SDF exhibited good antioxidant activity.

3.3 Hypoglycemia activity of U-SDF in vitro

Inhibition of the activity such as α-amylase and α-glucosidase is an important strategy to alleviate postprandial hyperglycemia (Li et al., 2021). When the concentration ranged between 0.5 mg/mL to 2.5 mg/mL, the α-amylase inhibition rate of U-SDF increased in a dose-dependent manner. When the concentration of the sample was 2.5 mg/mL, the α-amylase inhibition rate of U-SDF reached 27.34% ± 3.85%, while that of the control reached 18.69% ± 4.39% and acarbose reached 66.17% ± 3.37%. Although, the overall α-amylase inhibition rate by U-SDF was lower than the acarbose, which used in the clinic to treat T2DM, it was exhibited better inhibitory capacities than that of the control group at the same concentration (Figure 2a). The α-glucosidase inhibitory rate of U-SDF in the 0.5 mg/mL to 2.5 mg/mL range was shown in Figure 2b. The inhibitory effects of the U-SDF against α-glucosidase improved as the dosage increased. The U-SDF, which showed α-glucosidase inhibition of 46.03% ± 3.17% at 2.5 mg/mL concentration, exhibited better inhibitory capacities than the control, but was evidently weaker than acarbose. These indicated that U-SDF may be a potential inhibitor of α-amylase and α-glucosidase and exerted an effect on hypoglycemia *in vitro*, which broadens the application of *Lentinula edodes* in food industry.

3.4 The hypoglycemic effect of U-SDF in STZ-induced diabetic mice

Although the body weight was no significant change, food intake and water intake of diabetic mice significantly decreased compared with the DG after 3 weeks of treatments with U-SDF, and the change trend was positively correlated with the U-SDF concentration. They were reduced 16.16% (5.19 ± 0.35 g vs. 6.19 ± 0.51 g, $p < 0.01$) and 24.08% (11.70 ± 0.53 mL vs.15.41 ± 0.82 mL, $p < 0.001$) compared to the NG with the U-SDF dosage of 1000 mg/kg (Supplementary Fig. 1a-c). The urine output of diabetes mice was significantly reduced compared to the DG (Supplementary Fig. 1d). Moreover, it was noted that treatment with U-SDF for 3 weeks significantly reduced the fasting blood glucose and insulin levels, with maximal efficacy being observed in mice from U-S-1000 group, which exhibited a 42.33% (17.21 ± 1.79 mmol/L vs. 29.84 ± 2.12 mmol/L, $p < 0.001$) decrease in blood glucose and 29.57% (10.36 ± 0.71mU/L vs.14.71 ± 0.91 mU/L, $p < 0.001$) decrease in insulin levels relative to diabetic group, leading to significant improved the HOMA-IR ($p < 0.001$) and HOMA-IS ($p < 0.001$) compared with diabetic mice (Supplementary Fig. 1e-h). The OGTT data showed that the blood glucose of all the mice reached the peak after 30 min following glucose loading and had a decreasing trend within the next 90 min (Supplementary Fig. 1i). Compared to the control, the AUC of diabetic mice exhibited significantly higher postprandial glucose level (1978.77 ± 150.18 min·mmol/L vs. 466.08 ± 132.52 min·mmol/L, $p<0.001$), implying impaired glucose tolerance. U-SDF treatment can significantly reduce blood glucose as well as the AUC values compared to the saline-treated diabetic mice in a dose-dependent manner which exhibited a 23.20% decrease (1161.27 ± 157.67 min·mmol/L vs. 1978.77 ± 150.18 min·mmol/L, $p < 0.001$) in AUC level relative to DG (Supplementary Fig. 1j). These indicating that U-SDF treatment can reduce fasting blood glucose, improve the glucose tolerance, and increase insulin sensitivity.

3.5 Antioxidant activity of U-SDF in vivo

MDA content and SOD activity are often be used as the indicator to assess the severity of oxidative stress and cell injury. The MDA content in the serum (15.90 ± 1.12 nmol/mL vs. 8.44 ± 0.73 nmol/mL, $p < 0.001$) and liver (4.46 ± 0.46 nmol/mg prot vs. 1.87 ± 0.31 nmol/mg prot, $p < 0.001$) of diabetic mice exhibited an apparent increase, whereas activities of SOD in the serum (46.20 ± 8.20 U/mL vs. 111.85 ± 11.59 U/mL, $p < 0.001$) and liver (40.76 ± 8.42 U/mg prot vs. 96.04 ± 12.46 U/mg prot, $p < 0.001$) significantly decreased compared with the NG (Figure 3), this is consistent with previous study (Li et al., 2021). However, treatment with U-SDF significant decreased the MDA content and increased the SOD activity in the serum and liver of the diabetic mice in a dose-dependent manner. With the maximal efficacy being observed in mice receiving the U-SDF dosage of 1000 mg/kg, MDA content exhibited 36.1% and 38.57% decrease in the serum (10.16 ± 0.63 nmol/mL vs. 15.90 ± 1.12 nmol/mL) and liver (2.74 ± 0.27 nmol/mg prot vs. 4.46 ± 0.46 nmol/mg prot) relative to the mice of diabetic group, respectively. However, SOD activities showed

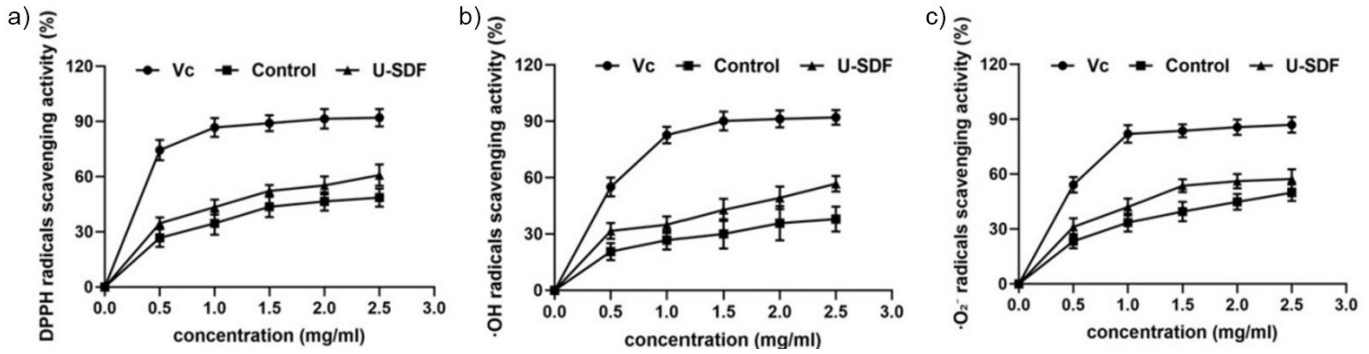

Figure 1. Scavenging effects of U-SDF from *L. edodes* on radicals. (a) Scavenging activity of DPPH radicals; (b) Scavenging activity of •OH radicals; (c) Scavenging activity of $\bullet O_2^-$ radicals.

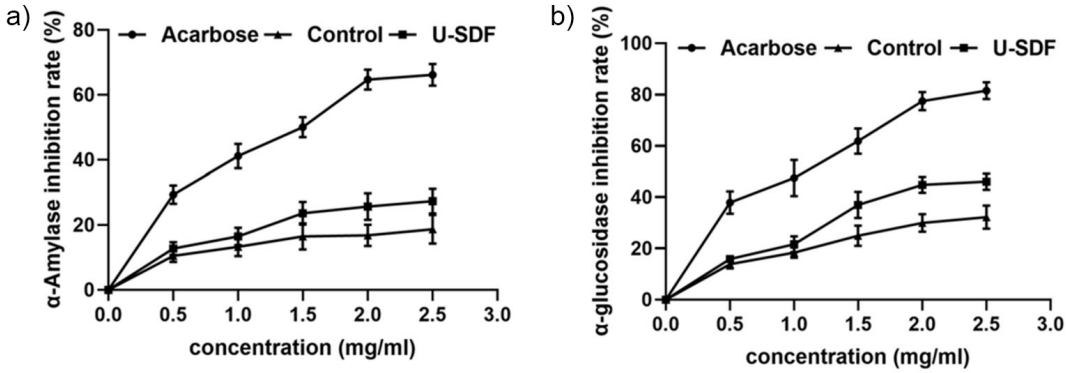

Figure 2. Hypoglycemic activity of the U-SDF. (a) inhibition ability on α-amylase activity; (b) inhibition ability on α-glucosidase activity.

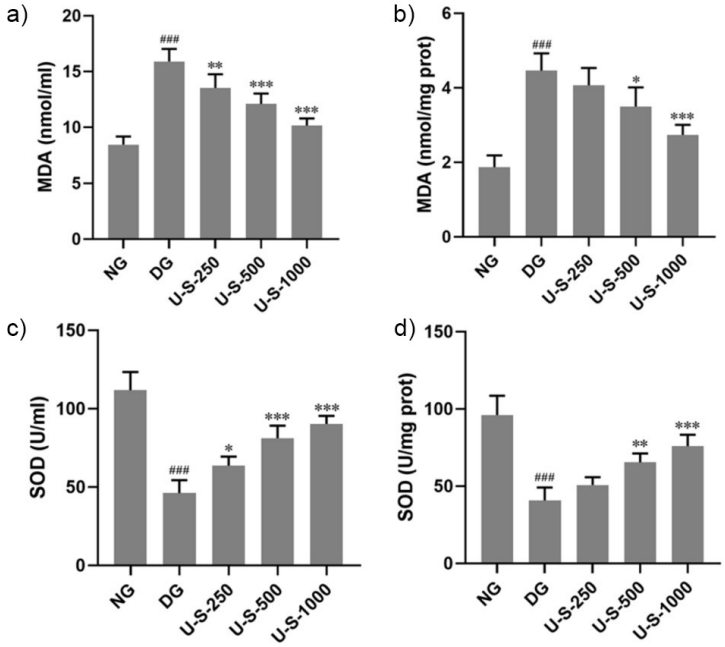

Figure 3. Effects of U-SDF treatment on (a) MDA content in serum, (b) MDA content in liver, (c) SOD activity in serum, (d) SOD activity in liver. ###, $p < 0.05$, vs. NG; *, $p < 0.05$, vs. DG; **, $p < 0.01$, vs. DG; ***, $p < 0.001$, vs. DG.

increase in the serum (90.30 ± 5.12U/mL vs. 46.20 ± 8.20U/mL) and liver (75.94 ± 7.42 U/mg prot vs. 40.76 ± 8.42 U/mg prot, $p < 0.001$), respectively. These results indicated that U-SDF can efficiently alleviate hyperglycemia-induced oxidative stress by reducing lipid peroxidation products and enhancing antioxidant enzymatic activity.

3.6 Histopathological observations

The importance of the endocrine pancreas lies in the fact that it plays a central role in the regulation of energy metabolism (Atkinson et al., 2020). The effects of U-SDF on pancreas in diabetic mice were evaluated as Figure 4a. Compared with the NG, the pancreatic cells of diabetic mice exhibited atrophy, and the pancreatic islets appeared irregular and the size of islets were significantly reduced. Treatment with U-SDF for 3 weeks significantly increased the islet amounts and restored the sizes of the islet cells. In addition, U-SDF treatment reduced STZ-induced pancreatic lesions, cell atrophy and cytoplasmic vacuolation. The liver is another important organ for regulating blood glucose (Zhang et al., 2019). The hepatic of normal mice showed regular cell shape and clear cell boundary, while the diabetic mice appeared cellular swelling and cytoplasmic vacuolation. In the group treated with U-SDF, hepatocyte hypertrophy and lipid vacuolation were significantly ameliorated compared to the NG mice (Figure 4b).

4 Discussion

In recent years, the high incidence of diabetes has increased the public's attention to healthy functional food (Chang et al., 2019). The positive effect of natural functional foods on human health and well-being is recognized (Pei et al., 2022). SDFs isolated from natural resources have attracted increasing attention on account of their multiple beneficial health effects. SDF not only plays an important role in maintaining dietary balance but also performs important physiological functions associated with the prevention of colon cancer and cardiovascular disease, as well as the lowering of cholesterol. Moreover, it reduces blood lipid content and delays the absorption of glucose by the small intestine, thus preventing diabetes (Gunness & Gidley, 2010).

L. edodes is widely used in food, medicine, health care products, and cosmetics industries due to the abundance of SDF (Zhang et al., 2012). Current SDF extraction technology involves the use of physical and chemical methods as well as biological enzymes and microbial fermentation techniques

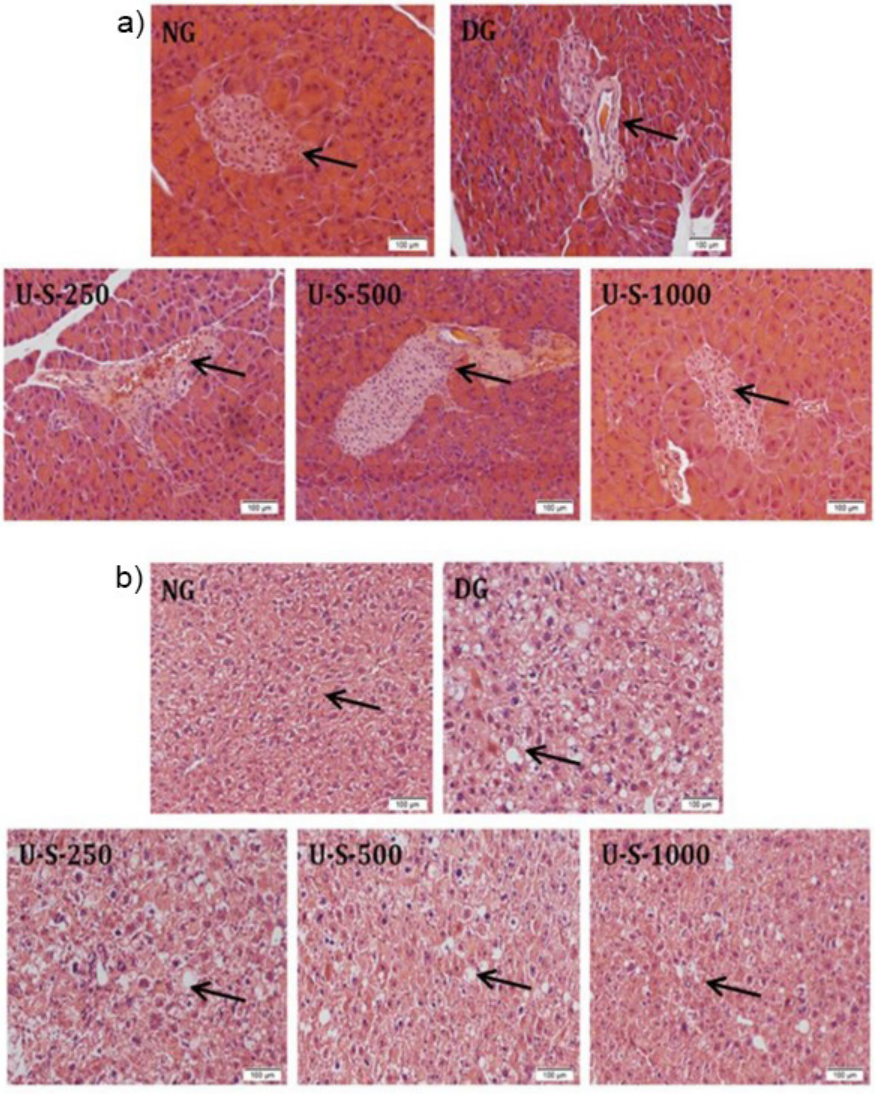

Figure 4. Histology observation of the pancreas and liver tissues after treatment with different concentration of U-SDF in diabetic mice. (a) pancreas; (b) liver. All sections were stained with H&E. The scale bars represents 100 μm.

(Karimi et al., 2020; Li et al., 2022b; Wang et al., 2022). Physical extraction is a green and environmentally friendly method that is widely used because it does not leave any chemical solvent residues (Wang et al., 2022). Hot water extraction, a physical technique, is most commonly used to extract SDF. SDFs extracted by this method show better physical and chemical properties, biological activities, low costs, and high extraction rates (Perez-Pirotto et al., 2022). Modification of DFs involves changing the ratio between IDF and SDF to improve the functional properties of DFs (Li et al., 2022a). Ultrasonic treatment is an attractive method for DFs modification due to its effectiveness and technical practicality. It can promote cavitation and destroys the structure of carbohydrates, loosens tissues, changes surface hydrophilicity, and causes mechanical and free radical scavenging effects (Chen et al., 2022). Following ultrasonic treatment, the SDF content of oats increased from 20.57% ± 0.30% to 31.60% ± 0.75%, particle size was reduced, and surface structure was loosened. Moreover, the number of pores in SDF was increased, causing it to become denser with a honeycomb shape. In addition, the hydrophilic and lipophilic groups of SDF are exposed, thereby significantly improving its hydration characteristics and oil-holding capacity (Niu et al., 2020). Therefore, ultrasonic was used to assist the hot-water method to extract SDF from *L. edodes* in this study. However, different extraction methods have great influence on the yield of SDF. Consequently, extraction methods must be further explored to obtain higher SDF yields from *L. edodes*.

Different raw materials and extraction methods may also lead to differences in the chemical properties, structural characteristics, and bioactivities of SDF (Dong et al., 2020; Wang et al., 2022). The DFs purified by alkali extraction method from defatted walnut flour showed better WHC and SC than those purified by enzymatic extraction method (Khan et al., 2018). The kiwifruit DFs prepared by enzyme and acid extraction methods showed better OHC and WHC than the DFs prepared by alkali extraction method (Wang et al., 2021). Therefore, it is necessary to evaluate the functional activity of U-SDF extracted in this study. The results showed that the WS, WHC, SC, and OHC of U-SDF were higher than that the SDF extracted using hot water method without ultrasonication. The DPPH, •OH, and $\bullet O_2^-$ radical clearance rates indicated that U-SDF exhibited better antioxidant activities. U-SDF also exhibited notable α-amylase and α-glucosidase inhibition activities. Treatment with U-SDF alleviated glucose and peroxidation metabolism disorders *in vivo*. Histological analysis indicated that U-SDF improved the oxidative tissue damage in diabetic mice. These results provided a theoretical basis for the development and utilization of SDF derived from *L. edodes.* In addition, although IDF has a rough texture and limited functional properties compared to SDF, its nutritional value is high. Therefore, future research may benefit by focusing on methods that may be utilized to modify grain IDFs, in order to better explore the potential application of DFs.

Conflict of interest

None.

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Supplementary Material

Supplementary material accompanies this paper.

Supplementary Fig. 1. Effects of U-SDF treatment on (a) body weight, (b) food intake, (c) water intake, (d) urine output, (e) plasma glucose, (f) insulin in mice, (g) OGTT, (h) AUC, (i) HOMA-IR and (j) HOMA-IS. ###, $p < 0.001$, vs. NG; **, $p < 0.01$, vs. DG; ***, $p < 0.001$, vs. DG.

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