


# Optimisation of ultrasonic-assisted hot-water extraction conditions of soluble dietary fibre from *Lentinula edodes* and analysis of its hypolipidaemic and anti-inflammatory properties in STZ-induced diabetic mice

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## ABSTRACT

Soluble dietary fibre (SDF) is well recognised for its remarkable effectiveness in promoting human health. This study utilised response surface methodology to evaluate the optimal conditions required to extract SDF (U-SDF) from *Lentinula edodes* via the ultrasonic-assisted hot-water method, and evaluated the hypolipidemic effects and anti-inflammatory effects of U-SDF. The optimal extraction conditions for U-SDF were ultrasonic power of 182 W, extraction time of 2 h, extraction temperature of 81 °C, and solid-liquid ratio of 1:24 (g mL<sup>-1</sup>). Under these conditions, the extraction rate of U-SDF reached 8.08%. U-SDF treatment significantly improved liver and kidney indices in diabetic mice, markedly reduced the levels of plasma triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and significantly increased the level of high-density lipoprotein-cholesterol (HDL-C) in a dose-dependent manner. U-SDF also improved adipose tissue injury in diabetic mice, significantly decreased the levels of cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and

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alleviated inflammation of the abdominal aorta. In conclusion, U-SDF from *L. edodes* is an excellent source of dietary fibres, which exhibit good hypolipidemic and anti-inflammatory activities, suggesting potential applications as a functional additive in diverse food products.

## KEYWORDS

*Lentinula edodes*, soluble dietary fibre, extraction optimisation, hypolipidemic, anti-inflammatory

## 1. INTRODUCTION

Dietary fibres (DFs) have been attracted much attention because of their remarkable effectiveness in promoting human health (Jackson et al., 2021). Grains such as wheat bran, oats, and corn husks are extremely high of DFs (Onipe et al., 2015; Kaur et al., 2019; Ratna et al., 2022), which are widely used in food processing to not only improve the utilisation rate of raw materials, but also increase the added value of agricultural products and improve economic efficiency. Vegetables and fruits are also major sources of DFs. DF-rich vegetables include soy beans, mushrooms, peas, and broccoli (Brumme et al., 2015; Lv et al., 2022; Rivas et al., 2022; Zhao et al., 2022). Extracting DFs from fruit pomace can improve the utilisation rate of fruit processing by-products (Pathania and Kaur, 2022). Moreover, seaweed is also a good source of DFs, with many studies analysing the physiological functions and physicochemical properties of DFs in seaweed (Huang et al., 2022; Yuan et al., 2023).

DFs can be classified into insoluble dietary fibres (IDF) and soluble dietary fibres (SDF) according to their solubility in water (Wang et al., 2022). SDF shows better physiological activities and superior processing properties than IDF, and it can prevent the occurrence of hypertension, diabetes, coronary heart disease, cardiovascular disease, and other chronic diseases (Guo et al., 2018). However, the content of SDF in total DFs is relatively low. Therefore, using effective extraction methods to improve the extraction rate of SDF may enhance the development of functional foods.

Different extraction methods affect the chemical and physical properties of SDF, thus affecting their functions and physiological characteristics. The physical method has the advantages of short extraction time and high yield and purity of SDF. As one of the most convenient physical extraction methods, hot water extraction is a simple, rapid, and environmental friendly way. Modification methods are generally used to increase the content of SDF (Guo et al., 2018). Ultrasonic extraction is a modification technique that exerts the effects of cavitation, vibration, crushing, and stirring generated via ultrasonic waves to reduce processing time and solvent usage (Chemat et al., 2016). Compared to traditional extraction methods, ultrasonic extraction confers the advantages of high speed, high extraction rate, low operational temperature, and less solvent use (Ahmad et al., 2015).

Type 2 diabetes mellitus (T2DM) is considered one of the most serious global health problems and is closely associated with cardiovascular disease and other complications (Shourabi et al., 2020). Hyperlipidaemia, which is defined as elevated levels of plasma TG, TC, and LDL but decreased level of HDL, is a prominent feature of T2DM (Chapman et al., 2011). Chronic low-grade inflammation is another important factor that is closely related to the pathogenesis of T2DM (Reinehr, 2019) with increased plasma concentrations of inflammatory



markers, such as IL-6, IL-1 $\beta$ , TNF- $\alpha$ , indicating the occurrence of insulin resistance (Tong et al., 2022). Therefore, it is an effective therapeutic strategy to prevent the occurrence and development of T2DM in reducing hyperlipidaemia, while improving inflammation.

*Lentinula edodes* is highly appreciated for its nutritional and nutraceuticals properties, being also considered as a novel source of DFs (Ziaja-Soltys et al., 2020). A previous study found that SDF extracted from *L. edodes* via the ultrasonic-assisted hot-water method (U-SDF) exhibited better physicochemical properties, antioxidant activities, and hypoglycaemic effects than that extracted without ultrasonication (Ni et al., 2023). However, it is unclear whether the SDF in *L. edodes* can alleviate inflammation and reduce hyperlipidaemia in diabetes. In this study, response surface methodology (RSM) was used to optimise ultrasonic-assisted hot water extraction method to extract SDF from *L. edodes*. Anti-inflammatory properties and dyslipidaemic functions of U-SDF were also estimated. Thus, the current study aimed to provide a theoretical basis for the development and utilisation of SDF derived from *L. edodes*.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of U-SDF from *L. edodes*

Air-dried *L. edodes* were cut into small pieces and crushed using a high-speed grinder (BJ-400A, China). The powder was then passed through a 60 mesh screen. The factors and levels of the single-factor experiment are listed in [Supplementary Table 1](#). The powder was dissolved in distilled water, and the solution was modified with an ultrasound instrument (CW2000, China). Subsequently, the solution was incubated in a water bath and filtered using a vacuum filter pump (SHZ-DIII, China). Trichloroacetic acid was added to the filtrate to remove the protein impurities. The filtrate was then centrifuged at 8,000 $\times$ g for 10 min, and concentrated using a rotary evaporator (SENCO, China) at 60 °C. The concentrated solution was incubated with 95% ethanol at 25 °C for 24 h, then centrifuged at 8,000 $\times$ g for 10 min to collect the precipitate. Finally, the precipitate was freeze-dried to obtain U-SDF for further analysis. The extraction yields were calculated using the following equation:

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

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

### 2.2. Response surface optimisation experiment

The ultrasonic power, extraction time, extraction temperature, and solid-liquid ratio, were employed as the uncoded variables to investigate the interaction between various factors, with U-SDF yield denoted as the response value  $Y$ . Response surface methodology and contour plots of the regression equation were used to visually interpret the relationship between the responses and experimental levels of each variable and the type of interactions between the two test variables.

### 2.3. Animal treatment

Male SPF grade C57/BL6J mice (20.0  $\pm$  2.0 g) were purchased from Pengyue Experimental Animal Breeding Co., Ltd. (Jinan, China). After adaptation for seven days, the diabetic mouse



model was established as described in our previous study (Ni et al., 2017). The diabetic group was divided into four groups: (1) diabetic control group (DG,  $n = 5$ ) with orally administered saline, (2) diabetic group administered U-SDF with the doses of  $250 \text{ mg kg}^{-1}$  (U-S-250), (3)  $500 \text{ mg kg}^{-1}$  (U-S-500), and (4)  $1,000 \text{ mg kg}^{-1}$  (U-S-1000) once daily for three weeks. The normal mice served as normal control (NG,  $n = 5$ ). All animal experimental protocols were approved by the Experimental Animal Ethics Committee of Xuzhou Medical University (approval number: L20210226457).

## 2.4. Biochemical parameters analysis and histological analysis

The mice were sacrificed at the end of the experiment and blood and tissue samples were collected. Blood samples were kept for 30 min at  $37^\circ\text{C}$ , then centrifuged at  $3,000\times g$  for 15 min at  $4^\circ\text{C}$  to obtain serum. The evidence suggests that dyslipidaemia and chronic low-grade inflammation are closely related to the pathogenesis of T2DM (Taskinen, 2003; Reinehr, 2019), so the lipid indicators (TC, TG, HDL-C, and LDL-C) and inflammatory factors (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ) were measured using the commercially available kits according to the manufacturer's instructions. Liver, kidney, adipose, and aorta tissues were fixed using 4% paraformaldehyde (Biosharp, China), then dehydrated with alcohol, and embedded in paraffin. All tissues were sectioned to a thickness of  $5 \mu\text{m}$  using a slicer (Leica, Germany) for staining.

## 2.5. Statistical analysis

Data were expressed as the mean  $\pm$  standard deviation. All data were statistically analysed using GraphPad Prism 8.0. Statistical comparisons among different groups were determined by one-way analysis of variance (ANOVA). Design Expert (Version 13) was used to perform response surface analysis. A value of  $P < 0.05$  was considered statistically significant.

# 3. RESULTS AND DISCUSSION

## 3.1. Single factor experimental analysis

The U-SDF yield increased significantly from 4.19% ( $\pm 0.29\%$ ) to 7.46% ( $\pm 0.36\%$ ) as ultrasonic power increased from 120 to 180 W. As the ultrasonic power continued to increase beyond 180 W, the extraction rate of U-SDF began to decrease (Supplementary Fig. 1a). The U-SDF yield also increased as the extraction time was extended from 0.5 to 2.0 h, reaching a maximum of 6.85% ( $\pm 0.23\%$ ) at an extraction time of 2.0 h before declining with increasing extraction time (Supplementary Fig. 1b). Moreover, the U-SDF yield increased from 4.82% ( $\pm 0.24\%$ ) to 6.77% ( $\pm 0.20\%$ ) as the extraction temperature increased from  $60^\circ\text{C}$  to  $80^\circ\text{C}$ , then began to decrease once the extraction temperature exceeded  $80^\circ\text{C}$  (Supplementary Fig. 1c). Finally, the U-SDF yield increased significantly from 3.71% ( $\pm 0.23\%$ ) to 6.08% ( $\pm 0.27\%$ ) as the solid-liquid ratio increased from 1:10 ( $\text{g mL}^{-1}$ ) to 1:20 ( $\text{g mL}^{-1}$ ), then began to decrease once the solid-liquid ratio exceeded 1:20 (Supplementary Fig. 1d).

## 3.2. Optimisation of extraction conditions by BBD

The values of the responses (U-SDF yield) at different experimental combinations for the coded variables are shown in Supplementary Table 3. By applying multiple regression analysis to the



experimental data, the response variable and the test variables were related using the following second-order polynomial equation:

$$Y = 7.70 + 0.1792X_1 + 0.1892X_2 + 0.1767X_3 + 0.1917X_4 - 0.04X_1X_2 + 0.09X_1X_3 + 0.0525X_1X_4 + 0.0625X_2X_3 + 0.01X_2X_4 - 0.0875X_3X_4 - 1.25X_1^2 - 1.78X_2^2 - 0.8697X_3^2 - 0.2598X_4^2 \quad (2)$$

Experimental data were evaluated using ANOVA, and the significance of the regression coefficients was evaluated using the corresponding *P*-values, which are presented in Table 1. ANOVA results of the quadratic regression model showed that the model was significant ( $F = 37.83$ ). The *F*-value (3.19) for lack of fit was not significant ( $P = 0.1377$ ) relative to pure error, while  $R^2$  value was 0.9742, indicating that the degree of fit of the model was good. The *F*-value showed that the solid-liquid ratio was the most significant parameter influencing the U-SDF yield, followed by the extraction time, ultrasonic power, and extraction temperature.

Contour and surface plots showed that the pairwise interaction between the four factors was insignificant, but exerted a synergic effect on U-SDF extraction. However, all factors exerted significant linear and quadratic effects on U-SDF extraction. Moreover, the contour plots indicated the following order of sequence of curve steepness: solid-liquid ratio > extraction time > ultrasonic power > extraction temperature (Fig. 1).

Table 1. Variance analysis of the response surface regression model

Source	Sum of squares	DF	Mean square	F-value	P-value
Model	29.39	14	2.10	37.83	<0.0001
X <sub>1</sub> -ultrasonic power	0.3852	1	0.3852	6.94	0.0196
X <sub>2</sub> -extraction time	0.4294	1	0.4294	7.74	0.0147
X <sub>3</sub> -extraction temperature	0.3745	1	0.3745	6.75	0.0211
X <sub>4</sub> -solid-liquid ratio	0.4408	1	0.4408	7.94	0.0137
X <sub>1</sub> X <sub>2</sub>	0.0064	1	0.0064	0.1153	0.7392
X <sub>1</sub> X <sub>3</sub>	0.0324	1	0.0324	0.5839	0.4575
X <sub>1</sub> X <sub>4</sub>	0.0110	1	0.0110	0.1987	0.6626
X <sub>2</sub> X <sub>3</sub>	0.0156	1	0.0156	0.2816	0.6040
X <sub>2</sub> X <sub>4</sub>	0.0004	1	0.0004	0.0072	0.9335
X <sub>3</sub> X <sub>4</sub>	0.0306	1	0.0306	0.5519	0.4698
X <sub>1</sub> <sup>2</sup>	10.15	1	10.15	182.94	<0.0001
X <sub>2</sub> <sup>2</sup>	20.46	1	20.46	368.70	<0.0001
X <sub>3</sub> <sup>2</sup>	4.91	1	4.91	88.43	<0.0001
X <sub>4</sub> <sup>2</sup>	0.4376	1	0.4376	7.89	0.0139
Residual	0.7769	14	0.0555		
Lack of fit	0.6902	10	0.0690	3.19	0.1377
Pure error	0.0867	4	0.0217		
Cor total	30.17	28			
R <sup>2</sup>	0.9742				
Adjusted R <sup>2</sup>	0.9485				
Predicted R <sup>2</sup>	0.8637				
Adeq precision	20.2774				
C.V. (%)	3.94				



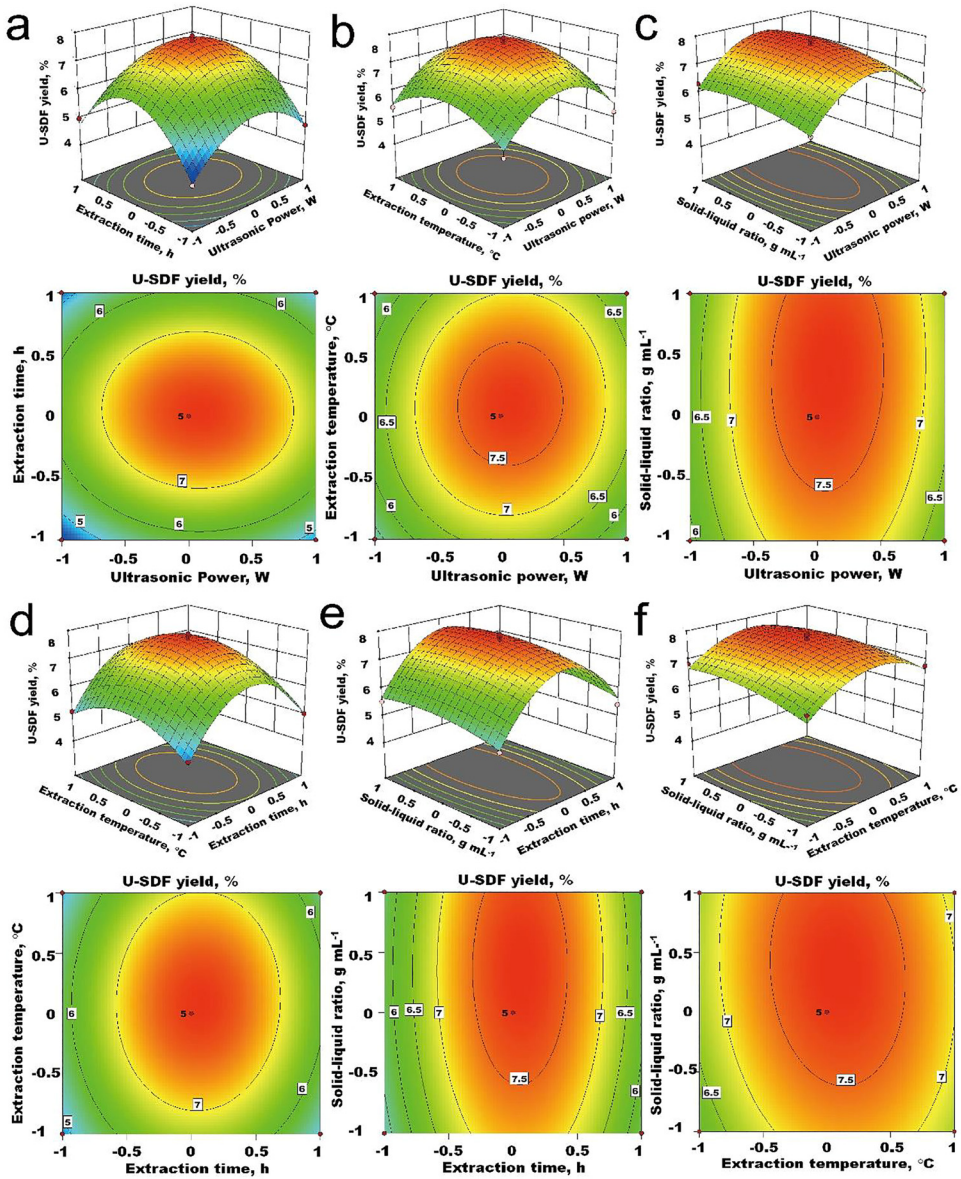


Fig. 1. 3D-response surface plot and contour plots for optimising extraction conditions, showing the effect of different extraction condition variables (independent factors) on U-SDF yield. (a) ultrasonic power and extraction time; (b) ultrasonic power and extraction temperature; (c) ultrasonic power and solid-liquid ratio; (d) extraction time and extraction temperature; (e) extraction time and solid-liquid ratio; (f) extraction temperature and solid-liquid ratio



Based on the analysis of response surface, the optimal conditions for extracting U-SDF were as follows: ultrasonic power of 182.44 W; an extraction time of 2.03 h, an extraction temperature of 80.89 °C, and a solid-liquid ratio of 1:23.64 (g mL<sup>-1</sup>). The theoretical value of the U-SDF yield was 7.76%. Subsequently, the extraction conditions were adjusted to ultrasonic power of 182 W, an extraction time of 2 h, an extraction temperature of 81 °C, and a solid-liquid ratio of 1:24 (g mL<sup>-1</sup>), which extracted a U-SDF yield of 8.08%.

Multiple alternative methods exist for extracting DF from *L. edodes*. For example, Xue et al. (2019) used the alkali-extraction method to extract SDF from *L. edodes* and achieved a maximum extraction rate of 6.09%. Conversely, a maximum extraction rate of 6.75% was achieved via the ultrasonic-assisted cellulase extraction of SDF from *L. edodes* stipe (Wan et al., 2023). Furthermore, Meng et al. (2017) adopted enzymatic hydrolysis to obtain an extraction rate of 14.33%. Thus, although the ultrasonic-assisted hot-water method employed in this study is not the most efficient SDF extraction method, it is simple to operate, environmentally friendly, and less expensive, which is highly significant for the development and utilisation of SDF from *L. edodes*.

### 3.3. Effect of U-SDF on organ index of liver and kidney organ indices in diabetic mice

The relative weights of the kidney and liver in DG increased by 17.09% ( $P < 0.05$ ) and 12.79% ( $P < 0.05$ ), respectively, compared with NG, indicating obvious swelling of the organs of diabetic mice (Table 2 and Fig. 2). In addition, the surfaces of the kidney and liver were coated with fat in

Table 2. Effect of U-SDF on the absolute and relative weights (g/100 g BW) of kidneys and livers in mice

Groups	Body weight, g	Absolute weight, g		Relative weight, g	
		Kidney	Liver	Kidney	Liver
NG	24.66 ± 0.99	0.41 ± 0.04	1.21 ± 0.05	1.65 ± 0.17	4.91 ± 0.21
DG	26.62 ± 1.48	0.53 ± 0.05	1.48 ± 0.04	1.99 ± 0.16 <sup>#</sup>	5.63 ± 0.33 <sup>#</sup>
U-S-250	25.14 ± 1.86	0.45 ± 0.03	1.29 ± 0.03	1.77 ± 0.18	5.20 ± 0.43
U-S-500	25.0 ± 1.12	0.42 ± 0.04	1.25 ± 0.03	1.70 ± 0.13	5.02 ± 0.31 <sup>*</sup>
U-S-1000	24.98 ± 1.56	0.42 ± 0.02	1.24 ± 0.02	1.67 ± 0.13 <sup>*</sup>	4.99 ± 0.28 <sup>*</sup>

<sup>#</sup>:  $P < 0.05$ , vs. NG; <sup>\*</sup>:  $P < 0.05$ , vs. DG.

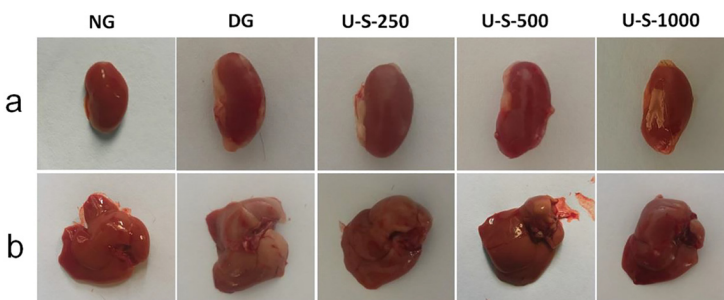


Fig. 2. Effects of U-SDF on the organ indices of diabetic mice. (a) kidney; (b) liver



DG (Fig. 2). Compared with DG, U-SDF treatment reduced organ swelling in a dose-dependent manner; kidney swelling was significantly reduced in the group treated with a dose of  $1,000 \text{ mg kg}^{-1}$  ( $P < 0.05$ ), and liver swelling was significantly relieved in the group treated with doses of 500 ( $P < 0.05$ ) and  $1,000 \text{ mg kg}^{-1}$  ( $P < 0.05$ ) (Table 2). Moreover, U-SDF treatment also significantly reduced the content of adipose tissue covering the organs of diabetic mice (Fig. 2).

### 3.4. Effect of U-SDF on the lipid profiles of diabetic mice

Increased plasma levels of TC, TG, and LDL-C and/or decreased levels of HDL-C are observed in diabetes (Guo et al., 2014). Moreover, reductions in the levels of serum lipids, either through drugs or diet, significantly decrease the risk of acquiring cardiovascular disease and other complications (Behradmanesh et al., 2013). Compared with NG, DG showed an increase of TC, TG, and LDL-C levels by 51.02% ( $P < 0.001$ ), 73.08% ( $P < 0.001$ ), and 61.03% ( $P < 0.001$ ), respectively, as well as a decrease of HDL-C levels by 35.90% ( $P < 0.001$ ). Compared to DG, U-SDF treatment markedly reduced the levels of TG, TC, and LDL-C (by 31.20% ( $P < 0.01$ ), 22.66% ( $P < 0.01$ ), and 33.82% ( $P < 0.001$ ) in U-S-1000, respectively) and significantly increased the level of HDL-C (by 22.28% ( $P < 0.01$  in U-S-1000)) in a dose-dependent manner (Fig. 3a–d). In addition, the cross-sectional area and volume of adipocytes in white adipose tissue (WAT), as well as the numbers of cell vacuoles in brown adipose tissue (BAT) were significantly higher in DG than in NG. Compared to DG, U-SDF treatment significantly decreased the cell size in WAT and the number of cell vacuoles in BAT. Thus, U-SDF treatment significantly improved dyslipidaemia in diabetic mice (Fig. 3e and f).

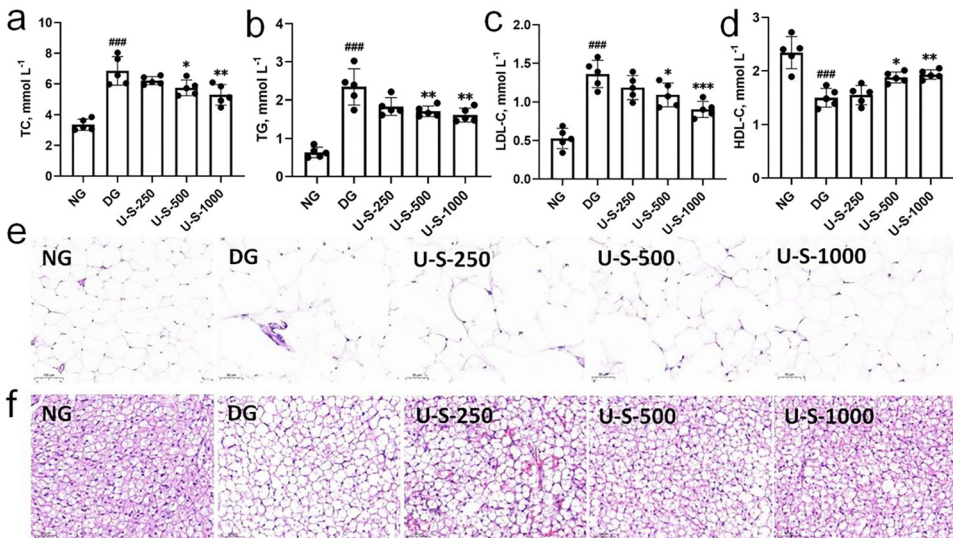


Fig. 3. Effect of U-SDF treatment on dyslipidaemia. (a) TC; (b) TG; (c) LDL-C; (d) HDL-C; (e) white adipose tissue; (f) brown adipose tissue. ###:  $P < 0.001$ , vs. NG; \*:  $P < 0.05$ , vs. DG; \*\*:  $P < 0.01$ , vs. DG; \*\*\*:  $P < 0.001$ , vs. DG



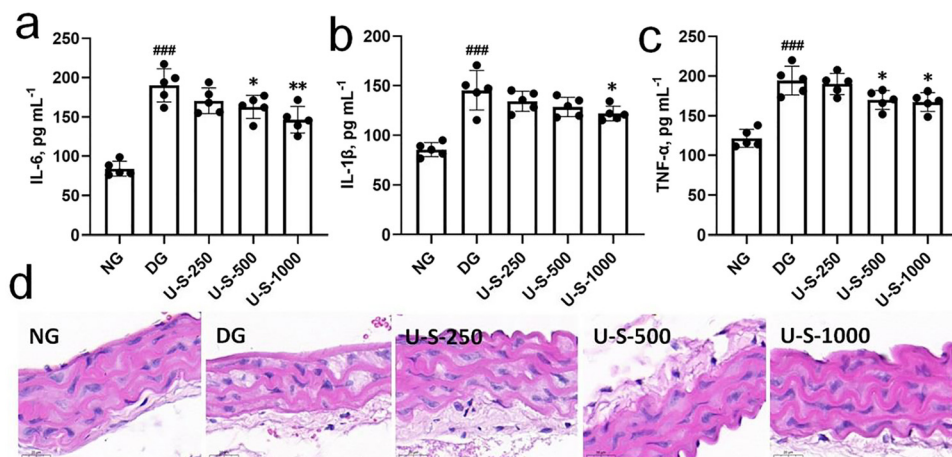


Fig. 4. Anti-inflammatory activities of U-SDF. (a) IL-6; (b) IL-1 $\beta$ ; (c) TNF- $\alpha$ ; (d) morphological observations of the abdominal aorta in mice. ###:  $P < 0.001$ , vs. NG; \*:  $P < 0.05$ , vs. DG; \*\*:  $P < 0.01$ , vs. DG

### 3.5. Effect of U-SDF on the anti-inflammation profiles of diabetic mice

Numerous studies have shown that chronic inflammation plays a crucial role in the development of T2DM and its complications by promoting insulin resistance or  $\beta$ -cell failure (Go et al., 2011). Therefore, targeted regulation of the inflammatory system is considered an important therapeutic strategy for T2DM (Reinehr, 2019). The effects of U-SDF on serum inflammatory cytokine levels are shown in Fig. 4. IL-6, IL-1 $\beta$ , and TNF- $\alpha$  levels in DG increased by 56.01% ( $P < 0.001$ ), 41.20% ( $P < 0.001$ ), and 37.47% ( $P < 0.001$ ) compared to NG, respectively. After three weeks of U-SDF treatment, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 levels significantly decreased in a dose-dependent manner by 22.99% ( $P < 0.01$ ), 16.06% ( $P < 0.05$ ), and 13.86% ( $P < 0.05$ ) in U-S-1000, respectively (Fig. 4a–c). Moreover, the endometrium of the abdominal aorta in NG mice was smooth and flat, with endothelial cells arranged neatly and without rupture, whereas that in DG mice was uneven, endothelial cells were swollen and disordered, and inflammatory cells were attached. After treatment with U-SDF, the degree of abdominal aortic inflammation improved, and endothelial cells were arranged more neatly than those in DG (Fig. 4d).

## 4. CONCLUSIONS

In this study, we first optimised the conditions for extracting SDF from *L. edodes* through ultrasonic-assisted hot-water extraction. The optimal extraction conditions were as follows: ultrasonic power of 182 W, an extraction time of 2 h, an extraction temperature of 81 °C, and a solid–liquid ratio of 1:24, which resulted in a U-SDF yield of 8.08%. However, different raw materials and extraction methods may lead to differences in the chemical properties, structural characteristics, and bioactivities of SDF obtained from *L. edodes*. Therefore, extraction methods must be further explored to obtain higher SDF yields. Second, we performed experiments to investigate the hypolipidaemic and anti-inflammatory effects of U-SDF in diabetic mice. U-SDF



significantly improved the liver and kidney organ indices of diabetic mice, significantly reduced TC, TG, and LDL-C levels, and increased HDL-C levels. In addition, U-SDF treatment significantly decreased the cell size of WAT and the number of cell vacuoles in BAT, significantly reduced the levels of serum inflammatory markers, and improved abdominal aortic inflammation. These results indicate that U-SDF can be used as an alternative functional food for alleviating diabetes and its cardiovascular complications through its hypolipidaemic and anti-inflammatory activities. Overall, *L. edodes* is an excellent source of SDF; thus, the findings of this study have important implications for the development and utilisation of SDF extracted from *L. edodes*.

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## SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at <https://doi.org/10.1556/066.2023.00128>.

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