



## Hypoglycemic effect of dietary fibers from bamboo shoot shell: An *in vitro* and *in vivo* study

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### ABSTRACT

Bamboo shoot shell (BSS) was enzymatically decomposed to yield insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and total dietary fiber (TDF), which were investigated for their hypoglycemic properties using *in vitro* and *in vivo* methods. The results indicated that SDF exhibited significantly higher glucose adsorbing capacity than those of IDF and TDF. Moreover, SDF showed similar inhibition potential against  $\alpha$ -amylase with acarbose. TDF displayed the greater capacities of delaying glucose diffusion and inhibition of  $\alpha$ -glucosidase than those of SDF. In the diabetic mice, after 4-week administration of BSS fibers or metformin, the blood glucose levels were significantly reduced and the oral glucose tolerance was improved. TDF and IDF hardly influenced the blood insulin level, while SDF could significantly increase blood insulin level. The results showed that BSS fibers could be a potentially available dietary ingredient in functional food.

### 1. Introduction

Dietary fibers (DFs) are carbohydrate polymers of plant origin, which escape digestion and absorption in the human upper GI tract (Brownlee, 2011). Based on whether it dissolves in the water, DF could be classified into soluble (SDF) and insoluble dietary fiber (IDF) with particular metabolic and physiological effects in humans (Nsor-Atindana et al., 2012). Increasing evidences confirm that dietary fiber intake is strongly associated with the prevention and treatment of certain chronic diseases, such as cardiovascular disease, obesity, diabetes and cancer (Benitez et al., 2011; Chuang et al., 2012; Li et al., 2016).

Diabetes mellitus (DM) is becoming one of the most important public health challenges and economic burden of the twenty-first century (Fernandes et al., 2016). Type 2 DM (T2DM), accounting for 90% of DM, occurs due to inefficient processing of insulin (Lysy et al., 2016). In the T2DM population, the development of insulin resistance (IR) and pancreatic  $\beta$ -cell dysfunction lead to persistent hyperglycemia in the body (Kokil et al., 2015). The existing treatments, including oral hypoglycemic drugs and insulin, could alleviate some of the complications

of T2DM but not able to control of this chronic disease at the population level. It was well reported that bioactive compounds extracted from various plant or marine resources exhibit significant effects in delaying and managing the onset of T2DM (Feng et al., 2018; Ramachandran et al., 2017; Wang et al., 2016b; Zhao et al., 2018a, 2018b). It was further stated that DFs could decrease the post-prandial serum glucose levels and exhibit promising hypoglycemic properties through retarding the diffusion of glucose, adsorbing glucose and inhibiting the activities of carbohydrate digestive enzymes (Ahmed et al., 2011; Arun et al., 2017; Chau et al., 2004b). The health benefits of DFs have caused increasing scientific interests, and hence encouraged food scientists to search for new sources of dietary fibers as food supplementation to provide added health benefit.

Bamboo shoot, a nutritious and healthy food with low fat and rich dietary fiber, has been consumed in many countries for a long history. The storage period of fresh bamboo shoots is short and thus most of them are processed to various forms such as canned food, fermented food and soft drinks (Satya et al., 2010). Bamboo shoot shell (BSS) is a main by-product of the bamboo shoot processing industry, and huge quantity of discarded BSS is causing considerable resource waste and

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environmental pollution. In recent years, dietary fibers extracted from various agroindustrial by-products, such as soybean residues (Chen et al., 2014), orange peels (Wang et al., 2015), cocoa shells (Nsor-Atindana et al., 2012) and rice bran (Wang et al., 2016a), were extensively studied. In our previous studies, BSS has been proved to be a cheap source for low-cost polysaccharide (Zheng et al., 2016) and dietary fiber (Luo et al., 2017) production, and the resulting carbohydrate polymers exhibit promising healthy effects.

In this study, BSS was enzymatically hydrolyzed to obtain soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) by using multi-enzyme method. The BSS fiber fractions, SDF, IDF and total dietary fiber (TDF, sum of SDF and IDF), were investigated for their hypoglycemic effects using *in vitro* and *in vivo* methods. As diabetes is multifactorial origin, the present study was designed to investigate the hypoglycemic potential of BSS fiber fractions by exploring the underlying mechanisms in terms of glucose adsorption capacity, glucose diffusion and dialysis retardation index (GDRI) and inhibition capacity of carbohydrate digesting enzyme ( $\alpha$ -amylase and  $\alpha$ -glucosidase) of BSS fiber fractions. Moreover, their potential hypoglycemic effect was evaluated in hyperlipidemia mice induced by high-fat diet and streptozotocin (STZ) injection.

## 2. Materials and methods

### 2.1. Materials and reagents

Fresh bamboo shoot shells were obtained from bamboo shoot processing factories in Fujian, China. The shells were washed and cut into small pieces, dried at 60 °C and crushed into BSS powder with 60-mesh screen.

Metformin hydrochloride sustained release tablets were bought from a local pharmacy (Beijing, China). The multiple enzymes purchased from Wolsen Biotechnology Co. (Xi'an, China).  $\alpha$ -Amylase,  $\alpha$ -glucosidase, acarbose, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (PNP-Gluc), and dinitrosalicylic acid (DNS) were obtained from Sigma Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade.

### 2.2. Preparation of dietary fiber fractions

The dietary fiber fractions were extracted from BSS powder according to previously reported method (Luo et al., 2017). Briefly, 200 g BSS powder was enzymatically hydrolyzed to obtain soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) by using multiple enzymes containing equal amount of cellulase (40 U/mg), protease (2000 U/mg) and amylase (2000 U/mg) at 40 °C for 1.6 h. The sum of resulting SDF and IDF is the total dietary fiber, named TDF.

### 2.3. Scanning electron microscopy (SEM) images

The surface and microstructure of SDF and IDF fractions were observed by SEM (SU8010 scanning electron microscope, Hitachi, Ltd., Tokyo, Japan) at an acceleration voltage of 15 kV. The dehydrated samples were placed on a specimen holder with double-sided conducting adhesive tapes and coated with a 10-nm gold layer. The micrographs were taken for each sample at magnification of 10,000 and 20,000, respectively.

### 2.4. Determination of glucose-adsorption capacity

Glucose-adsorption capacity of each dietary fiber sample was measured according to the reported method (Nsor-Atindana et al., 2012). Each dietary fiber sample (0.25 g, recorded as W) was mixed with 25 mL (recorded as V) of glucose solution at different concentration (0.5–50 mM, recorded as G<sub>1</sub>). The mixture was stirred and incubated in a thermostatically controlled water bath at 37 °C for 6 h, and then centrifuged at 4000 g for 15 min. The glucose content in 1 mL of the

supernatant was determined using glucose assay kits from Rongsheng Biotech Co., LTD (Shanghai, China) and recorded as G<sub>2</sub>. Each test was repeated three times and the glucose-adsorption capacity ( $\mu\text{mol/g}$ ) was calculated using the following equation:

$$\text{Glucose-adsorption capacity } (\mu\text{mol/g}) = (G_1 - G_2) / W \times V$$

### 2.5. Measurement of glucose diffusion and glucose dialysis retardation index

Glucose diffusion and dialysis retardation index (GDRI) was determined according to the previously reported method with minor modifications (Qi et al., 2016). Briefly, 0.2 g of dietary fiber sample was added into 10 mL of glucose solution (100 mM) and thoroughly mixed. The mixture was dialyzed against 200 mL of distilled water in a water bath at 37 °C using a dialysis membrane with a molecular weight cutoff of 12,000. After 20, 40, 60, 90, 120 and 180 min, the glucose content in 1 mL of the dialysate was measured using the glucose assay kit and recorded as G<sub>1</sub>. A control test was carried out without the addition of fiber sample, whose glucose content was recorded as G<sub>2</sub>. The GDRI was calculated through the following equation:

$$\text{GDRI } (\%) = 100 - [(G_1 / G_2) \times 100]$$

### 2.6. Measurement of inhibitory capacity of $\alpha$ -amylase

The  $\alpha$ -amylase inhibitory capacity was determined according to the reported method (De et al., 2013) with slightly modifications. Briefly, 100  $\mu\text{L}$  of different concentrations (range 0.2–1.0 mg/mL) of fiber sample or acarbose solution (positive control) were added to 1 mL of the assay mixture containing 0.02 M sodium phosphate buffer (pH = 6.9) and  $\alpha$ -amylase (0.83  $\mu\text{g/mL}$ ), incubated in a water bath at 37 °C for 10 min. This was followed by incubation at 37 °C for another 10 min after adding 200  $\mu\text{L}$  of potato starch solution (1% w/v). The reaction was terminated by adding 2 mL DNS and placing in a boiling water bath for 5 min. After cooling down to room temperature, the final mixture was diluted with 9 mL of distilled water and the absorbance measured at 540 nm and recorded as A<sub>1</sub>. The control without any fiber sample were also measured and recorded as A<sub>2</sub>. The results were expressed as % inhibition calculated using the formula:

$$\text{The } \alpha\text{-amylase inhibition rate } (\%) = (A_2 - A_1) / A_2 \times 100$$

The IC<sub>50</sub>, which is the concentration of the sample required to inhibit 50% of the enzyme was determined for each sample.

### 2.7. Measurement of inhibitory capacity of $\alpha$ -glucosidase

Based on the amount of *p*-nitrophenol released from PNP-Gluc, the inhibitory capacity of  $\alpha$ -glucosidase was measured (Hilala et al., 2015). The well-mixed reaction mixture contained 0.6 mL of phosphate buffer (0.1 M), 0.2 mL of  $\alpha$ -glucosidase (5U/mL) and 0.2 mL of different concentrations (range 0.2–1.0 mg/mL) of fiber sample or acarbose solution (positive control). The mixture was pre-incubated in a water bath at 37 °C for 10 min, and then was mixed with 0.4 mL of PNP-Gluc (20 mM). After 30 min of incubation at 37 °C, the reaction was stopped by adding 2 mL of 0.1 M of sodium carbonate. In the control tubes, the same amount of buffer was used to replace the fiber sample. The production of *p*-nitrophenol was quantified by measuring the absorbance at 405 nm, the values for the test and control tubes were recorded as A<sub>1</sub> and A<sub>2</sub> respectively. The inhibitory percentage was calculated by means of the following formula:

$$\text{The } \alpha\text{-glucosidase inhibition rate } (\%) = (A_2 - A_1) / A_2 \times 100$$

## 2.8. Animal experiments design

This investigation was performed on male Kunming mice ( $20 \pm 2$  g) purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China). The mice were free to access food and water and housed in stainless steel cages (4 mice per cage) at a controlled temperature ( $23 \pm 2^\circ\text{C}$ ) with a 12/12 h light-dark cycle and humidity of  $60 \pm 10\%$ . To protect the experimental animals used in this study, all the experimental procedures were executed in accordance with the EU Directive 2010/63/EU, and approved by the Animal Care Review Committee, Fujian Agriculture and Forest University, China.

After 1 week of acclimatization, the mice were used as diabetic models, which were induced by high-fat diet and streptozotocin (STZ) injection according to the previously reported method (Zheng et al., 2016). The mice were fed with high-fat diet (HFD, Feed formula: Basal diet 63.6%, cholesterol 1.2%, sodium salt 0.2%, egg yolk powder 10%, sucrose 15%, and lard 10%). After three weeks, mice were injected i.p. with STZ freshly dissolved in citrate buffer (pH 4.5) at a dose of 50 mg/kg body weight. Seven days later, fasting blood glucose was determined using the One-Touch Ultra blood glucose meter (LifeScan, Milpitas, CA, USA), and all the mice exhibited high blood glucose level ( $> 12.0$  mmol/L) which were considered to be qualified as diabetic model. The diabetic mice were further divided into 5 groups of 8 mice: a diabetic model control (DMC) group without intervention; a metformin-treated (Met) group treated with metformin (300 mg per kg bodyweight) (Suh et al., 2015); SDF group fed with basal diet mixed with 5% (w/w) SDF; a IDF group fed with basal diet mixed with 5% (w/w) IDF; a TDF group fed with basal diet mixed with 5% (w/w) TDF. The dose of fiber sample was decided according to our previous study (Luo et al., 2017), which indicated that the basal diet containing 5% of BSS fiber would not significantly affect the food intake of the experimental mice.

## 2.9. Measurement of blood glucose, OGTT and insulin levels

In the following 4 weeks, the experimental animals' general health status was monitored, and fasting blood glucose (BG) levels in the tail blood were measured every week using the One-Touch Ultra blood glucose meter. One day before the termination of the experiment, an oral glucose tolerance test (OGTT) was performed (Harazaki et al., 2014). After 8 h of fasting, the mice received a glucose load (2 g/kg body weight) orally, and blood samples were collected from tail vein at 0 (before glucose administration), 30, 60, 90 and 120 min after glucose load. On the last day of experimentation, the animals were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected and centrifuged at 6000 rpm at  $4^\circ\text{C}$  for 15 min to separate the serum. The insulin levels were determined using an insulin ELISA kit (Xinyu Biotechnology Co., Shanghai, China).

## 2.10. Statistical analysis

All data were expressed as means  $\pm$  standard deviation. Statistical analyses were performed using Data Processing System (DPS) statistical software. An ANOVA followed by a Duncan's multiple range test (DMRT) were used to evaluate treatment effects at significance level of  $p < 0.05$ .

## 3. Results

### 3.1. Preparation of dietary fiber samples

IDF and SDF were enzymatically hydrolyzed from BSS powder with the extraction rates of 56.21% and 8.67%, respectively. The extraction rate of SDF was significantly increased, which was consistent with the findings of a recent study (Xu et al., 2015), indicating that enzyme hydrolysis, especially for cellulase, could significantly increase the SDF

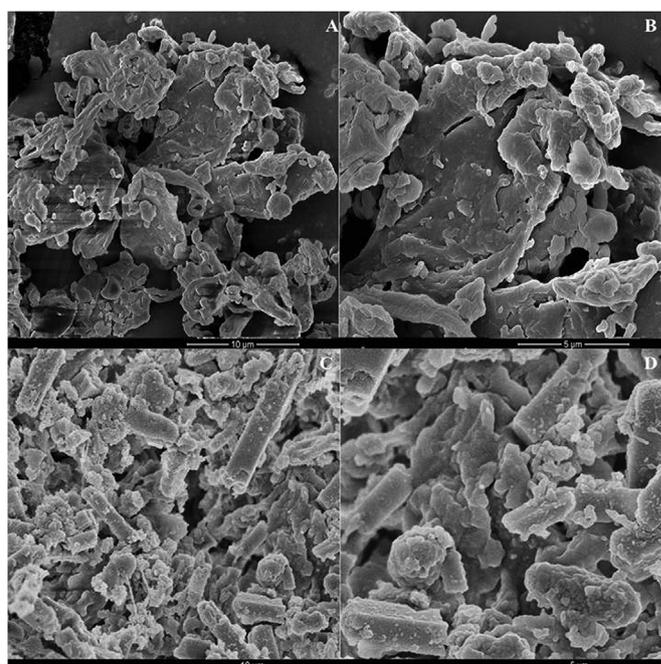


Fig. 1. Scanning electron micrographs of bamboo shoot shell insoluble dietary fiber (IDF), soluble dietary fiber (SDF) after multi-enzyme treatment. (A) IDF  $\times 10,000$ ; (B) IDF  $\times 20,000$ ; (C) SDF  $\times 10,000$ ; (D) SDF  $\times 20,000$ .

extraction rate by hydrolyzing insoluble fibers into soluble ones and releasing trapped soluble fiber. Finally, TDF containing both SDF and IDF was 64.88%. The resulting BSS fiber fractions, including IDF, SDF and TDF, were used for the further *in vitro* and *in vivo* investigation.

### 3.2. SEM images of fiber samples

Scanning electron micrographs of bamboo shoot shell IDF and SDF were shown in Fig. 1. Fig. 1A and B revealed that the IDF particles had an irregular and loose structure with a sheet-like appearance. The SDF particles (Fig. 1C and D) showed a rod-shape structure and a more regular morphology than the IDF particles. The residues from the grinding procedure were found on the surface of both SDF and IDF particles. Multi-enzyme processing caused a reduction of particles sizes by disrupting the chains, while IDF had a greater size of particle compared with that of SDF particle. The difference in the external and internal structures of fibers might have an impact on their *in vitro* and *in vivo* properties.

### 3.3. Glucose adsorption capacity

The BSS fibers were found to be effective in adsorbing glucose at different glucose concentrations (0.5–50 mM), and their glucose adsorption capacities were proportional to the glucose concentration (Fig. 2). Consistent to the fiber fractions isolated from cocoa shells (Nsor-Atindana et al., 2012), SDF exhibited significantly higher ( $p < 0.05$ ) glucose adsorbing capacity than those of IDF and TDF at different glucose concentrations. It was reported that the high viscosity of SDF could be an important factor for the entrapment of glucose molecules and thereby the delaying of glucose diffusion. The SEM images in Fig. 1 revealed that the enzymatic hydrolysis resulted smaller particle sizes and larger specific surface area of SDF than those of IDF, which might be another factor for a better glucose adsorption capacity (Ma and Mu, 2016). Generally speaking, there was no significant difference ( $p > 0.05$ ) was found between the glucose adsorbing capacities of IDF and TDF, due to the high content of IDF in TDF. Moreover, when the glucose concentration decreased to 5 mM, all of the BSS fibers could

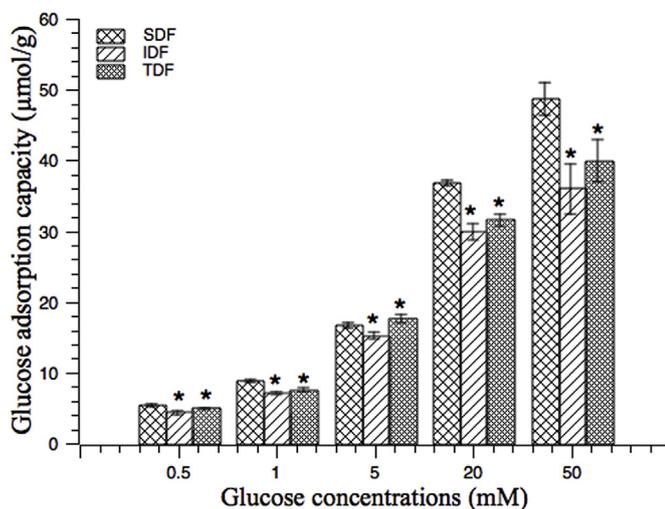


Fig. 2. Glucose adsorption capacity ( $\mu\text{mol/g}$ ) of the fibers at different concentrations of glucose. SDF: soluble dietary fiber; IDF: insoluble dietary fiber; TDF: total dietary fiber. Results are expressed as mean  $\pm$  SD ( $n = 3$ ). \* $p < 0.05$  compared with SDF.

still adsorb certain amount of glucose (range from 4.63 to 5.36  $\mu\text{mol/g}$ ), thereby keep the glucose concentrations in the small intestine at a relatively low level, which contributes to impede the postprandial hyperglycemia.

### 3.4. Glucose diffusion and glucose dialysis retardation index

As observed in Fig. 3A, the glucose contents in the dialysate among fiber samples were increased along with the time. The glucose contents in the dialysates of SDF, IDF and TDF ranged from  $13.37 \pm 0.26$  to  $17.02 \pm 0.21 \mu\text{mol}$  at 20 min and  $385.40 \pm 6.58$  to  $392.03 \pm 5.33 \mu\text{mol}$  at 180 min. Compared with the control, three fiber samples exhibited significantly inhibitory effects ( $p < 0.05$ ) against the movement of glucose into the external liquid across the dialysis membrane during the first 120 min, while no significant difference ( $p < 0.05$ ) was noted at 180 min.

GDMI has been used as a useful *in vitro* index to indicate the effect of fibers on the delay in glucose absorption in the gastrointestinal tract (Lopez et al., 1996). According to the retardation in glucose diffusion, GDRI for the various fiber samples were calculated and shown in Fig. 3B. It was noted that GDRI maximal values were reached after 20 min for SDF (38.07%), IDF (33.33%) and TDF (47.63%). It was reported that the retardation of glucose diffusion by dietary fibers might be influenced by several factors, including the adsorption capacities of the fibers to the glucose, the viscosity of soluble fibers and the opening network structure of insoluble fiber particles which contributed to the entrapment of glucose (Qi et al., 2016). In this study, TDF showed greater capacity of delaying glucose diffusion than those of SDF and IDF, and it could be explained by that TDF combined the factors from both SDF and IDF to adsorb and entrap the glucose at the same time.

### 3.5. Inhibitory capacities of $\alpha$ -amylase and $\alpha$ -glucosidase

Alpha-amylase and  $\alpha$ -glucosidase are known as key enzymes in starch breakdown and absorption. It has been well documented that the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is strongly related to the significant decrease of the blood glucose level after meal (Gabbia et al., 2017), and thus this approach has been proved as an efficient and cheap treatment for T2DM (Porte, 2001). The capacities of BSS fibers to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase were measured by incubating these enzymes with an increasing concentration (0.2–1.0 mg/mL) of fiber samples. Acarbose, a popular anti-diabetic drug which could efficiently

inhibit the glycoside hydrolase, was used as a positive control. As shown in Fig. 3 C,D, all BSS fibers could inhibit the *in vitro* activities of the two enzymes in a dose-dependent manner, which was consistent to the previously reported studies (Gabbia et al., 2017; Roy et al., 2011). The  $\text{IC}_{50}$  values, the concentration of the sample required to inhibit 50% of the enzyme, were calculated and listed in Table 1.

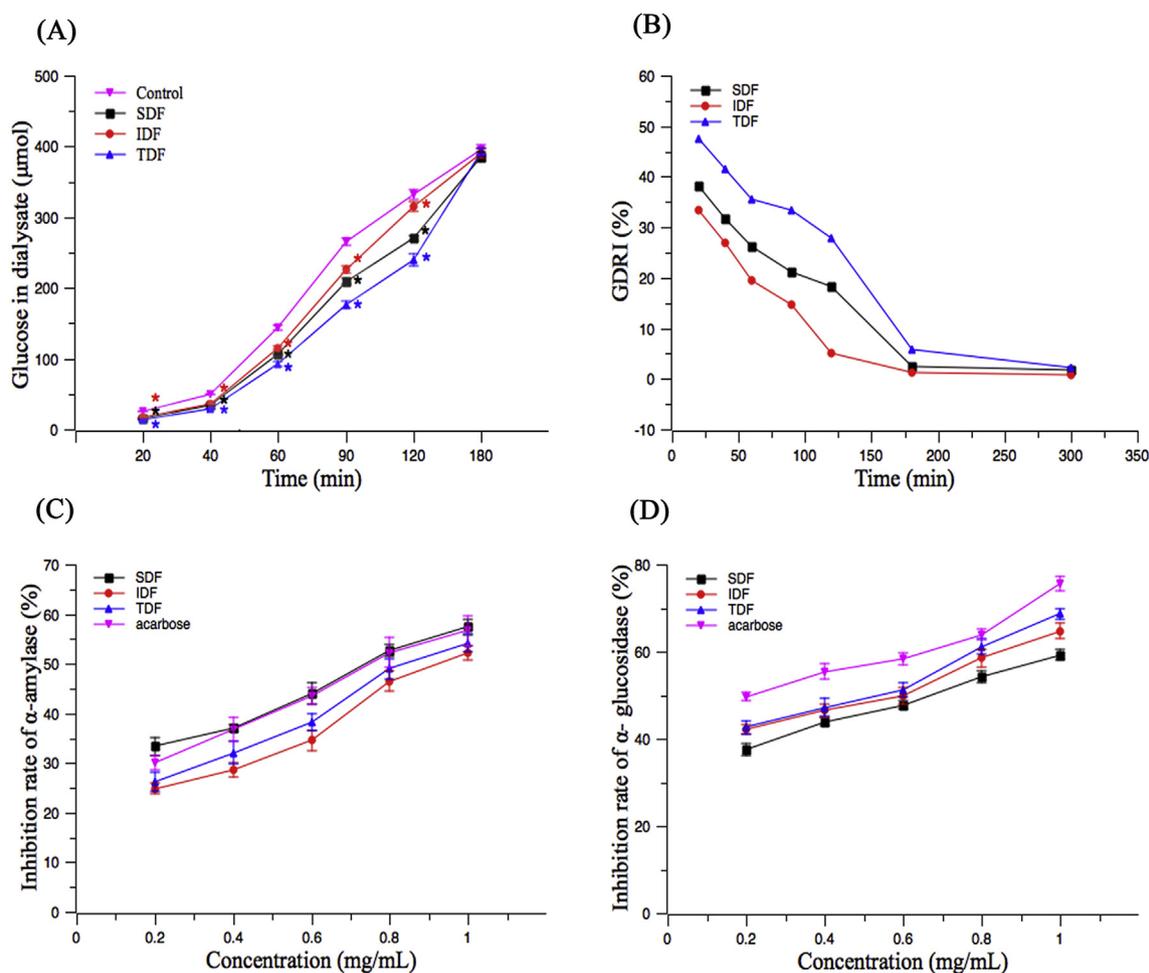
For the inhibition of  $\alpha$ -amylase, compared to the  $\text{IC}_{50}$  value of the standard inhibitor acarbose (0.78 mg/mL), those of SDF, IDF and TDF were 0.76 mg/mL, 0.95 mg/mL and 0.88 mg/mL, respectively. The result indicated that SDF exhibited similar inhibitory capacity of  $\alpha$ -amylase compared with that of acarbose. For the inhibition of  $\alpha$ -glucosidase, the  $\text{IC}_{50}$  values of SDF (0.65 mg/mL), IDF (0.52 mg/mL) and TDF (0.48 mg/mL) were all higher than that of acarbose (0.24 mg/mL). The result indicated that BSS fibers have promising inhibitory potencies against  $\alpha$ -amylase and  $\alpha$ -glucosidase, which might be attributed to their capacities in entrapping starch and enzymes by the opening fiber network, and even inhibiting the enzyme directly by some inhibitors presenting on the fiber surface (Qi et al., 2016).

### 3.6. *In vivo* hypoglycemic effects of BSS fibers in mice

The *in vivo* hypoglycemic effects of BSS fibers were further investigated on the diabetic mice. As shown in Fig. 4A, there was no significant effect on the bodyweights in the groups with or without intervention at the end of the experimental period. The effects of BSS fibers administration on blood glucose levels in diabetic mice were shown in Fig. 4B. The diabetic mice exhibited the expected hyperglycemia with the blood glucose levels ranging 12.78–13.33 mmol/L, which were two folds higher than that from healthy mice. The diabetic mice in MDC group without any treatment remained a high blood glucose level of  $13.87 \pm 0.65 \text{ mmol/L}$  in the end of experiment. However, the 4-week BSS fiber (5%, w/w) or metformin (300 mg per kg bodyweight) administration exhibited a significant effect on the reduction of blood glucose in the diabetic mice. Versus the MDC group, the blood glucose levels in mice from the SDF, IDF, TDF and Met groups were significantly reduced by 30.1%, 27.3%, 38.2% and 41.1% at the end of experiment, respectively. As revealed in the results, TDF containing both SDF and IDF showed the greatest hypoglycemic effect, which was more promising than that of supplementation with SDF or IDF alone. The results indicated that TDF supplementation (5%, w/w) has similar hypoglycemic effect as metformin administration (300 mg per kg bodyweight). More important, TDF was prepared from natural plant resource, and therefore no side-effect caused to the diabetic mice was observed during the experiment.

The oral glucose tolerance test (OGTT) was performed on the last day of experiments. As shown in Fig. 4C, blood glucose levels of mice were constantly changed at all time-points during the OGTT. After the mice received a glucose load (2 g/kg body weight) orally, the concentrations of blood glucose in all experimental mice peaked at 30 min. Compared to the MDC group, the blood glucose levels in the fiber- and metformin-treated groups declined significantly ( $p < 0.05$ ) at 60, 90 and 120 min after glucose loading. The results suggest improved oral glucose tolerance in diabetic mice due to BSS fiber.

The effects of BSS fibers or metformin on serum insulin levels in diabetic mice are shown in Fig. 4D. In our study, the diabetic mice were induced by HFD and STZ injection, and the latter caused damage to the beta cells in the pancreas. Therefore, the insulin level in all the diabetic mice was significantly decreased compared with that of healthy mice. After 4-week administration of BSS fibers or metformin, only SDF administration could improve the decrease in insulin level ( $p < 0.05$ ). There was no significant difference found among the other groups, indicating that the hypoglycemic effects of IDF, TDF and metformin were not mainly contributed by increasing insulin release from the beta cells in the pancreas.



**Fig. 3.** Effects of bamboo shoot shell fibers on glucose diffusion (A), GDRI (B), and inhibition rate of  $\alpha$ -amylase (C) and  $\alpha$ -glucosidase (D). GDRI: glucose diffusion and dialysis retardation index; SDF: soluble dietary fiber; IDF: insoluble dietary fiber; TDF: total dietary fiber; control: without any fiber sample. Results are expressed as mean  $\pm$  SD ( $n = 3$ ). \* $p < 0.05$  compared with control or acarbose.

**Table 1**

IC<sub>50</sub> values for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition of BSS fibers and acarbose.<sup>a,b</sup>

	IC <sub>50</sub> value (mg/mL)	
	$\alpha$ -amylase	$\alpha$ -glucosidase
SDF	0.76 $\pm$ 0.05	0.65 $\pm$ 0.04*
IDF	0.95 $\pm$ 0.11*	0.52 $\pm$ 0.02*
TDF	0.88 $\pm$ 0.07	0.48 $\pm$ 0.02*
Acarbose	0.78 $\pm$ 0.08	0.24 $\pm$ 0.02

\* $p < 0.05$  compared with acarbose.

<sup>a</sup> BBS: bamboo shoot shell; SDF: soluble dietary fiber; IDF: insoluble dietary fiber; TDF: total dietary fiber.

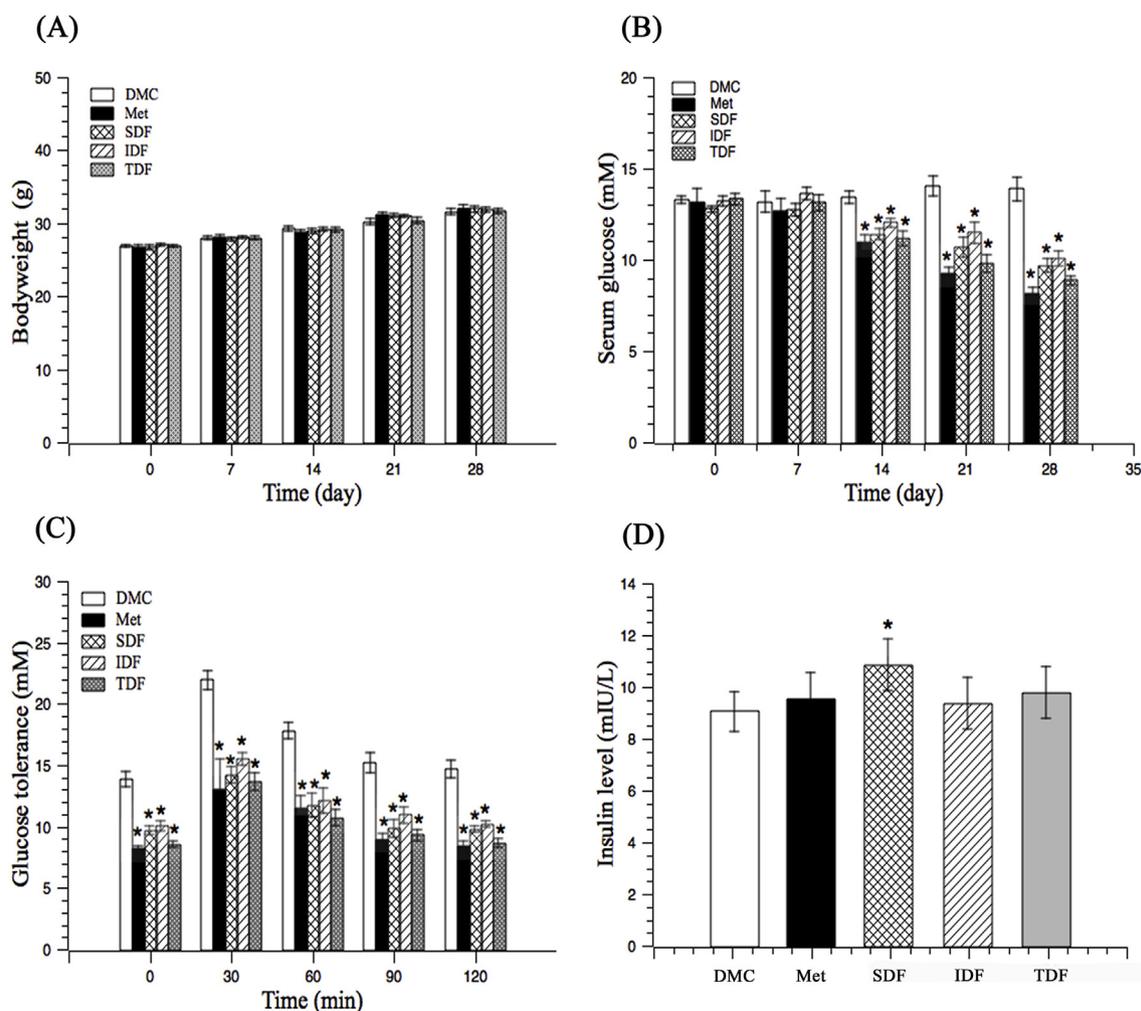
<sup>b</sup> Results are expressed as mean  $\pm$  SD ( $n = 3$ ).

#### 4. Discussion

To effectively manage T2DM, pharmacological treatments are usually required to combine with dietary interventions, which could augment their actions (Farag and Gaballa, 2011). It is strongly recommended that sufficient amount of dietary fibers should be supplemented in the diets for the individuals at risk for or diagnosed with diabetes. The dietary fibers prepared from cereals, fruits and vegetables, such as rice bran (Qi et al., 2016), carrot (Chau et al., 2004a) and starfruit (Chau et al., 2004b), have been investigated for their capacities of decreasing the risk of chronic diseases caused by

hyperglycemia. Similar to these fiber-rich foods, bamboo shoot is another commonly consumed healthy food well-known for its high content of dietary fiber. In the present study, we used bamboo shoot shells, a cheap and abundant resource of dietary fiber, for the production of three fiber fractions, including soluble (SDF), insoluble (IDF) and total dietary fiber (TDF).

As supposed by Ou et al. (2001), the role of dietary fiber in lowering postprandial serum glucose could be explained by the following pathways and thus investigated with the appropriate *in vitro* methods: the first one was to adsorb glucose and keep the glucose concentrations in the small intestine at a relatively low level; the second one was to increase the viscosity of the small intestinal content and retard the diffusion of glucose; and the third one is to inhibit the activities of carbohydrate digestive enzymes and postpone the release of glucose from starch. The *in vitro* experiment results revealed that all three fiber samples could exhibit favorable glucose adsorbing capacity, retardation of glucose diffusion and inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase at various degrees, which contributes to impede the postprandial hyperglycemia. SDF exhibited significantly higher ( $p < 0.05$ ) glucose adsorbing capacity than those of IDF and TDF at different glucose concentrations. It was consistent to the previously reported results that viscous soluble dietary fibers could exhibit greater potential on glucose adsorption (Babio et al., 2010; Vuksan et al., 2009). Moreover, SDF exhibited promising capacity of  $\alpha$ -amylase inhibition (IC<sub>50</sub> = 0.76 mg/mL), which is comparable to that of the standard inhibitor acarbose (IC<sub>50</sub> = 0.78 mg/mL). TDF exhibited the greater capacities of delaying



**Fig. 4.** Effects of the fibers on bodyweight (A), serum glucose (B), glucose tolerance (C) and insulin level (D) of diabetic mice. DMC: diabetic mice without intervention; Met: diabetic mice treated with metformin (300 mg per kg bodyweight); SDF: diabetic mice fed with basal diet mixed with 5% (w/w) SDF; IDF: diabetic mice fed with basal diet mixed with 5% (w/w) IDF; TDF: diabetic mice fed with basal diet mixed with 5% (w/w) TDF. Results are expressed as mean  $\pm$  SD of 8 mice in each group. \* $p < 0.05$  compared with DMC.

glucose diffusion and inhibition of  $\alpha$ -glucosidase than those of SDF, indicating that the insoluble fibers presented in TDF could also contribute and further enhance these functions by adsorbing the glucose or entrapping enzymes due to their loose structure and rough surface.

Based on the *in vitro* results, the BSS fibers were revealed to have potential in postponing the release of glucose from starch, delaying the rate of glucose absorption, and then decreasing the concentration of postprandial serum glucose. To further investigate the potential hypoglycemic effect of BSS fibers, diabetic mice were induced using HDF and STZ injection for the *in vivo* experiment. The successfully induced diabetic mice exhibited the expected hyperglycemia with high blood glucose and low insulin levels mainly due to the damage of the beta cells in the pancreas. Metformin, a widely used oral medicine for diabetes, was used as a positive control in this *in vivo* investigation. Metformin works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues, but not by increasing insulin release from the beta cells in the pancreas. After 4-week administration of BSS fiber or metformin, the typical diabetes symptom of the treated mice was significantly relieved at various degrees. The blood glucose levels in diabetic mice were significantly reduced and the oral glucose tolerance was improved, which indicated BSS fibers could help to improve the status of the diabetic mice, in spite of the fact that the effect was not as good as metformin. However, metformin together with TDF and IDF showed no effect on the serum insulin levels, while

SDF could significantly increase serum insulin level, which could be due to its capacity of stimulation of insulin release from the beta cells in the pancreas. In our previous study, BSS fibers administration could effectively decrease the body weight gain and improve the lipid metabolism disorderly situation in hyperlipidemic mice (Luo et al., 2017), which were believed to be able to enhance their anti-diabetic effects. The results from *in vitro* and *in vivo* experiments indicated that soluble and insoluble BSS fibers exhibited the hypoglycemic effects through complicated and different pathways. Although SDF exhibited more promising capacities on glucose adsorption,  $\alpha$ -amylase inhibition and stimulation of insulin secretion, the result from animal experiment revealed that TDF administration showed the greatest hypoglycemic effect in the diabetic mice. Further investigations are needed to better understand the mechanism of hypoglycemic effect of the BSS fibers. Based on the existing results, TDF prepared from bamboo shoot shell can be used effectively as a safe diet supplementation to control postprandial hyperglycemia.

#### Conflicts of interest

The authors declare no conflict of interests.

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