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Structure characterization and hypoglycemic effects of dual modified resistant starch from indica rice starch

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ABSTRACT

Hypoglycemic effects of indica rice resistant starch (IR-RS) were investigated. We prepared IR-RS using a method that combined physical modification and enzyme modification, and the RS content was 47.0%. Differential scanning calorimetry – thermal gravimetric analysis showed that IR-RS have higher enthalpy and less loss of mass than single modified RS, heat-moisture RS and native starch. Scanning electron microscopy revealed that IR-RS displayed more compact spatial structure. IR-RS products displayed a mixture of B-and V-type x-ray diffraction patterns and the cyrstallinity was 51.0%. IR-RS significantly affected body weight, blood glucose, organ indices and serum lipid levels. These results demonstrated that dual modification changed the structure of indica rice starch and affected its digestibility as well as the blood glucose levels of the diabetic mice who consumed it.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a common endocrine and metabolic disease that is caused by an absolute or relative lack of insulin in the blood, resulting in hyperglycemia and diabetes, which often goes on to cause fat and protein metabolic disorders (Shaw, Sicree, & Zimmet, 2010). The complications of T2DM have been associated with obesity, oxidative damage, dysfunction and eventual organ failure (Huang et al., 2005; Sharma, Balomajumder, & Roy, 2008). As the social and economic environment in China has improved, the incidence of diabetes (especially T2DM) has rapidly increased. Although many drugs can treat and control T2DM, including biguanides, sulfonylureas and α-glucosidase inhibitors, the long-term use of anti-diabetic drugs can result in symptoms of hypoglycemia, liver and kidney dysfunction and other adverse reactions (Posuwan et al., 2013). Therefore, the best approach is not to focus on the treatment of diabetes but rather to focus on how to prevent diabetes from occurring.

Resistant starch (RS) functions as a new food ingredient that has a low glycemic index. RS is termed an anti-digested starch, which refers to the fraction of the starch that cannot be digested in the small intestine and is partially fermented in the large intestine to produce short-chain fatty acids and other products (Haralampu, 2000). RS is characterized by a smaller molecular structure with a length of 20–25 glucose residues (linear polysaccharides that are connected by hydrogen bonding). RS has physiologic effects that are similar to those of dietary fiber (Bjorck & Asp, 1994). RS can affect body weight and energy balance (Losel and Claus, 2005) and increase lipid excretion to reduce calorie intake and decrease the serum lipid levels. RS itself contains almost zero calories (Alphons, 1998) and, when used as a low-calorie food additive, can control weight effectively. The ingestion of RS can decrease insulin secretion and control postprandial blood glucose to prevent diabetes (Weickert & Mohlig, 2005). RS can also lower the intestinal pH and promote the absorption of zinc, calcium and magnesium ions (Yonekura & Suzuki, 2005). The food and agriculture organization (FAO) listed RS as a dietary fiber that can be used for the prevention of T2DM (Devries, 2004) in 1990. Therefore, studies examining the preparation and function of RS are becoming increasingly important.

At present, RS can be prepared by physical, chemical and enzymatic modification. Hydrothermal treatments and annealing are physical modifications of starches that involve treating the starches at a moisture content of 30% and temperatures of 100 °C for 30 min, then keeping them at 70 °C for 12 h (Leea, Shinb, Kim, Choi, & Moon, 2011). Acid-alcohol treatment involves the chemical modification of starch with acids that are suspended in alcohol, which has the advantage of high granular recovery and

Abbreviations: IR-RS, indica rice resistant starch; DMT, dual modification-treated; SMT, single modification-treated; HMT, heat-moisture-treated; LG, low-dose group; MG, middle-dose group; HG, high-dose group; PC, positive control; MC, model control; NC, normal control.

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control of molecular degradation coupled with minor changes in granular structure (Robyt, Choe, Hahn, & Fuchs, 1996). Although the chemical method is available for use for industrial purposes, it may be accompanied by safety problems. Previous study (Zhang & Jin, 2011) has reported that the hydrolysis of maize starch with pullulanase can increase the RS content to 44.7%.

Although the three most commonly used raw materials in the starch industry are corn, wheat and potatoes, there has been an increased market demand for rice starch and its derivatives. Most studies on RS have focused on RS derived from potato, wheat, maize and sorghum sources. However, few studies have focused on RS prepared from rice starch. China is the largest rice producer and consumer in the world. Due to its low price and high RS content, indica rice could be the best candidate for high RS production. We prepared RS from indica starch using a new method that combines α-amylase, pullulanase and heat-moisture treatment. In this study, we optimized the reaction parameters in order to increase RS yield, and used in vitro digestion tests, structure detection and animal experiments to study the anti-digestibility, structure and hypoglycemic effects of IR-RS.

2. Materials and methods

2.1. Materials

Normal indica rice starch was obtained from Golden Agriculture Biotech Co., Ltd. Roche blood glucose meter and blood glucose test strips was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Pullulanse (1150 μ/g), heat-stable α-amylase (1400 μ/g) and amyloglucosidase (3300 μ/g), streptozotocin were purchased from Sigma–Aldrich Co., LLC (St. Louis, USA). Glimepiride Tablets was purchased from Chongqing Kangkeer Pharmaceutical Co., Ltd. (Chongqing, China). Other chemicals and reagents were analytical grade.

2.2. Preparation of resistant starch

2.2.1. Preparation of dual modification-treated (DMT) starch

Indica rice resistant starch (IR-RS) was prepared according to the methods described by Sievert and Pomeranz (1989) and Xu et al. (2012) with some modification. Indica rice starch (10 g, 25% w/v) was gelatinized with distilled water by stirring for 30 min at 80 °C and a pH of 6.0. The starch was treated with α-amylase in pH = 5.5, temperature = 80 °C, a reaction time of 40 min and an α-amylase level of 6 μ/g. Pullulanse was then added at 4 μ/g at a pH of 4.5 and a temperature of 46 °C for 12 h. The mixture was cooled to room temperature and then stored at 4 °C overnight in a refrigerator. The tempered starch was suspended in absolute ethanol and then shaken for 15 min. The ethanol was removed by centrifugation (8000 rpm, 15 min, 4 °C) and the procedure outlined above was repeated twice. The residue was then washed twice using distilled water. The washed starch was then treated in a boiling water bath for 90 min. The starch obtained was dried at 60 °C for 24 h and ground to pass a 200 mesh sieve for further analyses.

2.2.2. Preparation of single modification-treated (SMT) starch

SMT starch was prepared in the same manner as DMT starch, with the exception of α-amylase treatment.

2.2.3. Preparation of heat-moisture-treated (HMT) starch

HMT starch was prepared in the absence of enzyme. Indica rice starch (10 g, 25% w/v) was gelatinized with distilled water by stirring for 30 min at 80 °C and a pH of 6.0. After gelatinization, the indica rice starch was treated in a boiling water bath for 90 min and then dried at 60 °C for 24 h and ground to pass a 200 mesh sieve for further analyses.

2.3. RS determination

RS was determined according to the procedure outlined by Goñi, García-Diz, Mañas, and Saura-Calixto (1996) with a slight modification. RS (0.5 g) with distilled water (10 ml) was digested with excessive α-amylase (pH 5.8, 30 min, 100 °C). The reaction mixture was then cooled to room temperature for 30 min and centrifuged at 8000 rpm for 8 min. The resulting residue was collected and washed twice using distilled water. Each residue was then dispersed in 6 ml of a 2 mol/L KOH solution and stirred for 30 min at ambient temperature. The pH was adjusted to neutral using a citrate buffer solution and was then adjusted to 4.4 using a 2 mol/L HCl solution. Excess amyloglucosidase was added and the mixture was heated in a water bath at 60 °C for 45 min. The samples were centrifuged (8000 rpm, 8 min) and the supernatants were collected. The residues were resuspended in 20 ml of distilled water and the centrifugation was repeated. The supernatants were combined with the water washes and adjusted to a volume of 100 ml using distilled water. The reducing sugar contents were determined using the DNS method.

2.4. Anti-enzymatic activity of resistant starch

Each sample (5 g) was pregelatinized with distilled water (45 ml) by stirring for 30 min at 80 °C. The starch was treated with excess α-amylase and placed in a thermostatic water bath at 85 °C (Qiao, Mu, & Jia, 2009) at reaction times of 2, 6, 12, 24 and 36 h, and the reducing sugar contents in the solutions were determined.

2.5. Scanning electron microcopy (SEM)

RS were observed under a SEM (JSM-6490LV, Rigaku, Japan). Dried, finely ground samples were mounted on an aluminum stub with double-sided tape and coated with a thin film of gold. The samples were then observed and photographed at an accelerating voltage of 3 KV.

2.6. X-ray diffraction (X-RD)

A D/MAX2500 V (Rigaku, Japan) X-ray diffraction was used. The starch samples were equilibrated in a saturated relative humidity chamber for 24 h at ambient temperature and the starch powders were then scanned at a rate of 5°/min from 4° to 60° with a step time of 2 s. The relative crystallinity was calculated as the ratio of the crystalline areas and amorphous regions of x-ray diffractograms.

2.7. Differential scanning calorimetry – thermogravimetric (DSC-TGA) analysis

The gelatinization characteristics and thermogravimetric properties of the products were measured and recorded using differential scanning calorimetry – thermogravimetric (SDT Q600, TA instrument, USA) analysis. Approximately 5 mg of anhydrous starch samples were added to an aluminum DSC pan which was then sealed, reweighed and heated from 25 to 600 °C at 10 °C/min. An empty pan was used as a reference.

2.8. Animals experiments

2.8.1. Animals

Forty-eight male and forty-eight female SCXK (su) 2009-001 mice (SPF mouse) were purchased from Suzhou Science and Technology Co., Ltd. The experimental protocol was approved by the Animal Experiment Committee of China, in accordance with the

Table 1
Starch hydrolysis rate and RS content (%).

	RS content	2 h	6 h	12 h	24 h	36 h
Native starch	2.52[a] ± 1.647	25.7[a] ± 0.907	37.5[a] ± 1.23	45.3[a] ± 1.74	51.3[a] ± 1.07	53.7[a] ± 0.958
HMT starch	15.3 ± 1.256	12.2 ± 1.54	14.0 ± 1.78	15.3 ± 1.79	17.3 ± 0.770	18.2 ± 0.615
SMT starch	35.2[a] ± 1.073	6.55[a] ± 0.929	7.12[a] ± 1.09	7.55[a] ± 0.784	8.02[a] ± 0.890	8.02[a] ± 1.28
DMT starch	47.0[a] ± 1.232	3.17[a] ± 1.02	4.53[a] ± 0.938	5.17[a] ± 0.603	5.19[a] ± 1.24	5.19[a] ± 1.04

RS content (%) = Glucose content × 0.9/Starch weight ×100%.
Starch hydrolysis rate (%) = reducing sugar contents × 0.9/Starch weight ×100%
Each result represents the mean values ± standard deviation (n = 10).
[a] Indicates $p < 0.01$ as compared to the HMT starch.

National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

2.8.2. Streptozotocin (STZ)-induced diabetic mice

During the experiments, the mice were housed in an approved laboratory animal facility and maintained with controlled temperature (25 °C) and lighting (12 h light/dark cycle) for a 7-day adaptation period. The mice were fasted for 12 h and administered STZ (100 mg/kg) buffered in cold sodium citrate by intraperitoneal injection. Seven days after STZ injection, mice showing elevated fasting blood glucose levels were randomly assigned to different treatment groups.

2.8.3. Hypoglycemic effects of RS in diabetic mice

There were eight animals per cage and six groups (LG (low-dose group), MG (middle-dose group), HG (high-dose group), PC (positive control), MC (model control) and NC (normal control)). The mice in the PC group were given hypoglycemic agents (Glimepiride Tablets, 4 g/kg BW) and the mice in the MC and NC group were given 4 g/kg distilled water by gavage. The mice in the LG, MG and HG groups were administered 2, 4 and 8 g/kg DMT IR-RS aqueous solutions, respectively, once daily for 4 weeks by gavage. Each group had free access to food and water. The water bottles were filled twice daily and the animals were weighed once per week.

2.8.4. Anatomy of mice

Blood was collected through the eyes and rapidly centrifuged to obtain serum. The serum was stored at −20 °C for later determination of total serum cholesterol (TC), serum triacylglycerol (TG) and serum high-density lipoprotein cholesterol (HDL-C). The livers, kidneys and spleens were rapidly excised, rinsed in ice-cold saline, and weighed.

2.9. Statistical analyses

Origin 7.5 (Origin Lab Corporation, USA) statistical software was used by the first author of this study. All experimental data were expressed as the means ± SD. The differences between the test subjects and model controls were evaluated using Student's t-test.

3. Result and discussion

3.1. Anti-enzymatic activity of RS

The anti-enzymatic activity and RS content of the four kinds of starch samples are summarized in Table 1. Under the different conditions of preparing RS, the RS content of DMT starch was significantly higher (47.0%) than that of the other two samples. Some authors have reported a phenomenon of increased RS using a different preparation method (Htoon et al., 2010; Miao, Jiang, Zhang, Jin, & Mu, 2011; Ozturk, Koksel, & Ng, 2011). However, the RS content of their products was lower than that of our products.

With increased enzymatic hydrolysis time, the hydrolysis rate of the samples increased. The hydrolysis rate of the DMT starch was significantly less than that of the other two samples because after dual enzyme treatment with α-amylase and pullulanase, the starch molecules are hydrolyzed to a short, straight-chain. This chain is linked together by hydrogen bonds into a tight double-helical structure thereby forming a new crystalline structure and reducing the binding sites of the starch molecule with the enzyme making it difficult for the enzyme to be degraded. The decreased hydrolysis rate of RS in the in vitro starch digestibility assay has likely been previously explained to result from the fact that more short amylose may reduce the ability of starch to be digested by enzymes (Zhang, Sofyan, & Hamaker, 2008). The DMT therefore has strongly resistant enzymatic properties.

3.2. Scanning electron micrographs

Scanning electron micrographs of the granules of native and treated starch are provided in Fig. 1. The native indica rice starch granules had smooth surfaces and irregular polygonal shapes with no evidence of cracks under the SEM. However, after treatment with the different methods, the granular shapes changed. The surface of the HMT samples appeared to have an irregular, rough, non-uniform morphology and their structures were relatively loose. The surface of the SMT samples appeared to have large particles and a three-dimensional structure with a smooth surface. In comparison, the DMT starch granules featured a dense, compact and sheet-like structure that was less smooth than the native starch, but the DMT starch granules still maintained a relatively complete particle structure. This structure is attributed to the recrystallization of the gelatinized starch, amylase and amylopectin molecules that were rearranged through hydrogen bonding to move closer to one another, causing the re-composition of the mixed-dimensional crystal beam and thus maintaining the integrity of the starch and increasing the degree of crystallinity of the resistant starch. This reduced the action of the enzymatic sites and enhanced its resistance to digestion. This finding is also in agreement with the findings of Shi and Gao (Shi & Gao, 2011).

3.3. X-ray diffraction pattern and relative crystallinity

The X-ray diffraction patterns of the starches that underwent the different treatments are presented in Fig. 2. There are three different types of crystal structures: A-type starch crystallites, B-type starch crystallites and V-type starch crystallites. A-type starch crystallites is mainly cereal starches, such as corn, wheat and rice starch, with strong reflections at 15°, 17°, 18° and 23°; B-type starch crystallites is mainly starch tubers, fruits such as potato and banana starch, with strong reflections at 5.6°, 17°, 22° and 24°; V-type starch crystallites is generally related to amylase-lipid complex, with strong reflections at 14.5° and 19.5°. Native indica rice starch showed a typical A-type pattern with strong reflection at 2θ = 15.2°, 17.0°, 18.0°, and 23.1°. The heat-moisture-treated starch X-ray diffraction patterns were characteristic of the B- and V-types, with characteristic diffraction peaks appearing at approximately 2θ = 7°, 12°, 19° and 22°. Hydrothermal treatment changes the crystal

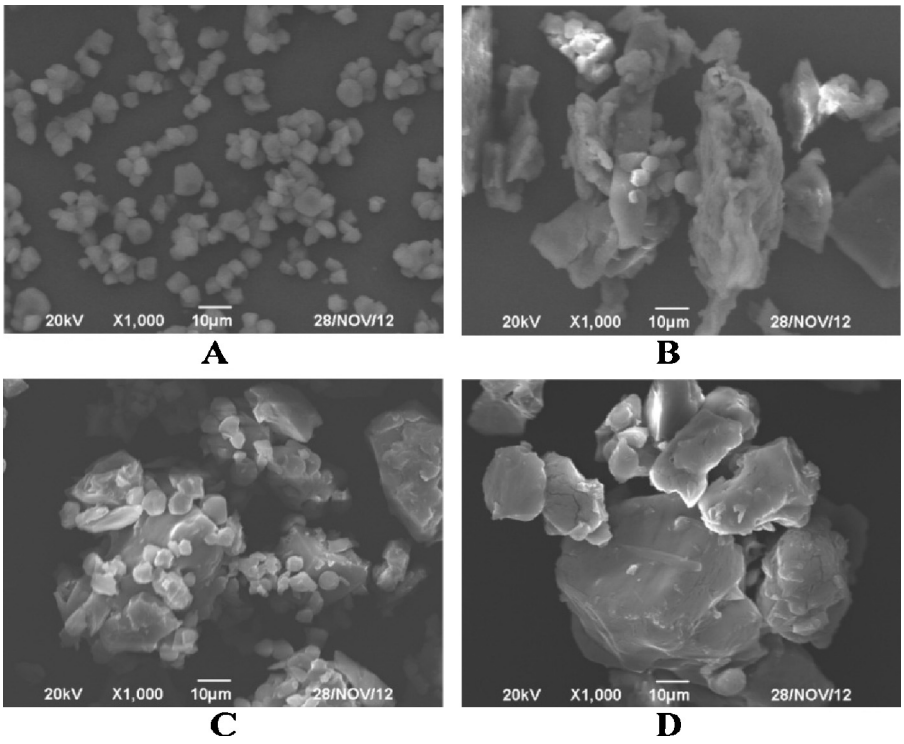

Fig. 1. Scanning electron micrographs of the native and treated starches: (A) native starch, (B) heat-moisture-treated starch, (C) single modification-treated starch, and (D) dual modification-treated starch.

pattern from A to B, as has been previously reported (Simsek & El, 2012). The diffraction pattern obtained for single-enzyme-treated starch appeared to be altered from the A-type to a combination of the A- and V-types (reflections at $2\theta = 8.03^\circ$, 12.6°, 15.1° and 19.9°). The X-RD traces of the dual-enzyme and heat-moisture-treated starches showed loss of reflection intensities at $2\theta = 15^\circ$, 18° and 23° and strengthened reflection intensities at $2\theta = 7^\circ$, 12°, 14°, 19°, 20° and 22°, suggesting a decline in A-type crystalline packing and an increase in B- and V-type crystalline packing.

The crystallinities of the native, heat-moisture-treated, single modified and dual modified starches based on X-RD were 18.1%, 20.2%, 27.0% and 51.0%, respectively. Compared with the native starch, the crystallinities of the heat-moisture-treated and single modified starches were decreased, but the crystallinity of the dual modification-treated starch was increased. In general, the degree of crystallinity can be attributed to crystal size, amount of crystallinity, orientation of the double helices and extent of interaction between the double helices (Song & Jane, 2000). When the dual modification method was used to prepare RS, the two enzymes (α-amylase and pullulanase) co-hydrolyzed the starch chain into a certain straight-chain length. The straight-chain then went on to form a double helix in the regenerative process. The heat-moisture process caused this structure to be closely arranged in an orderly manner and improved the crystallinity of the RS, therefore making it greatly resistant to amylolysis.

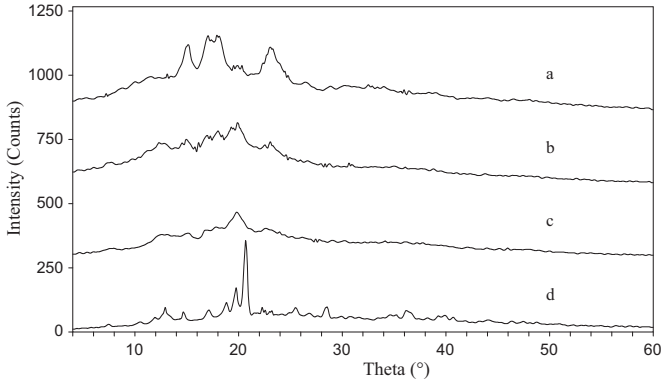

Fig. 2. X-ray diffraction patterns: (a) native starch, (b) heat-moisture-treated starch, (c) single modification-treated starch, and (d) dual modification-treated starch.

3.4. Thermal and thermogravimetric characteristic analyses

The thermal and thermogravimetric parameters of the native, HMT, SMT and DMT starches were analyzed using DSC-TGA and are summarized in Table 2. Table 2 shows that the native, HMT, SMT and DMT starches have endothermic peaks between 133 °C and 194 °C, 201 °C and 214 °C, 203 °C and 232 °C, and 207 °C and 235 °C, with enthalpies (which are the processes of starch crystals dissolving) of 37.1 J/g, 42.3 J/g, 51.3 J/g and 58.7 J/g, respectively. The increase of $T_o - T_c$ indicate that internal changes between each different resistant starches during the different treatment may lead to the formation of crystallites and the amounts of double helices with different stabilities. The increase in ΔH may reflect the fact that the DMT starch has greater amounts of doubles helices or stronger interactions between the starch chains within the crystalline domains of the starch. At 214 °C, the native starch has a large exothermic peak, which indicates severe decomposition of the starch, and a weight loss of 72.8%. The HMT, SMT and DMT starches also have large exothermic peaks at 255 °C, 267 °C and 295 °C, respectively.

TGA is widely used to evaluate the relationship between weight loss and temperature of starch because of their simplicity and effective information provide by a simple thermogram. Compared with the other three starches, the DMT starch lost much less weight, indicating that the crystal types are different. Similar study was found in Teramato, Motoyama, Yosomiya, and Shibata (2003) who used the TGA method found that there was difference in thermal resistance between amylase and amylopectin in corn starch.

Table 2
Thermal and thermogravimetric characteristics of the starches.

	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Phase transition temperature (°C)	TG (%)
Native starch	133[a] ± 1.06	158[a] ± 1.06	194[a] ± 1.41	37.1[a] ± 1.70	214[a] ± 1.714	72.8[a] ± 1.10
HMT starch	201 ± 1.35	205 ± 1.43	214 ± 1.85	42.3 ± 1.45	255 ± 1.40	50.1 ± 0.914
SMT starch	203[b] ± 1.40	223[a] ± 1.28	232[a] ± 1.03	51.3[a] ± 0.821	267 ± 0.811	45.7[b] ± 1.43
DMT starch	207 ± 1.01	226 ± 1.72	235[a] ± 1.41	58.7[a] ± 1.41	295 ± 1.23	36.4[a] ± 1.26

Each result represents the mean values ± standard deviation ($n = 10$).
[a] Indicates $p < 0.01$.
[b] Indicates $p < 0.05$ as compared to the HMT starch.

These results indicate that the DMT starch is able to maintain its crystal structure below 207 °C and has more heat stability than the other starches.

3.5. Effects of RS on body weight and blood glucose levels

Before STZ injection, after one week of STZ injection and 4 weeks of treatment with the different doses of DMT IR-RS, the body weights and blood glucose values of each group of mice were evaluated and compared to the values at the beginning of the experiment. The experimental data were analyzed and the results are as follows.

Fig. 3 shows the changes in body weight in each group of mice before and after the experiment. Before the STZ injection, all mice showed no significant difference in body weight. After the STZ injection, the mice under normal conditions grew larger, while the mice in the model group showed a decrease in body weight. After four weeks of feeding the mice the RS solution (LG, MG and HG) and hypoglycemic agents (PC), mice suffering from hyperglycemia showed increased body weight, with the HG and PC groups showing a significant increase as compared to the model control group ($P < 0.01$). No obvious behavioral changes were observed. Thus, RS prepared using the DMT method can relieve the weight loss caused by hyperglycemia.

Before the STZ injection, all mice showed no significant difference in body glucose (Fig. 4). After STZ injection, the levels of fasting blood glucose in the positive control, model control and IR-RS groups were significantly higher (the glucose levels in about 25 mmol/L) than those in the normal control group, and no significant difference between STZ injected groups, this indicating that a diabetic mouse model had been successfully established.

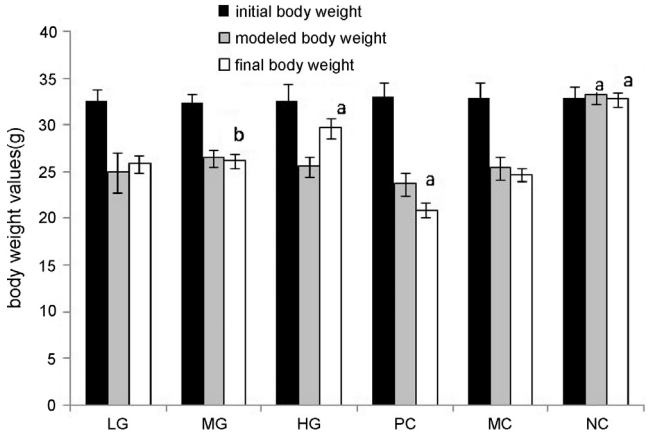

Fig. 3. Comparison of the body weights of experimental mice. NC, normal control group (distilled water, 4 g/kg BW); MC, model control group (distilled water, 4 g/kg BW); LG, low-dose group (DMT IR-RS, 2 g/kg BW); MG, middle-dose group (DMT IR-RS, 4 g/kg BW); HG, high-dose group (DMT IR-RS, 8 g/kg BW); and PC, positive control group (glimepiride solution, 4 g/kg BW). In addition to the oral administration, all mice were fed with the normal columnar diet. Each result represents the mean values ± standard deviation ($n = 10$). [a]Indicates $p < 0.01$ and [b]indicates $p < 0.05$ as compared to the MC.

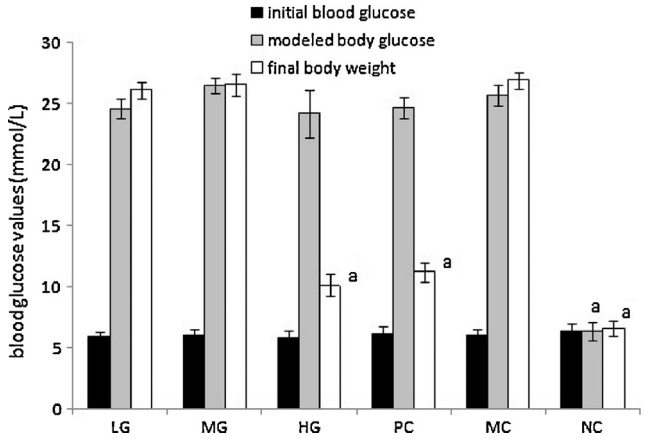

Fig. 4. Comparison of the body weights of experimental mice. NC, normal control group (distilled water, 4 g/kg BW); MC, model control group (distilled water, 4 g/kg BW); LG, low-dose group (DMT IR-RS, 2 g/kg BW); MG, middle-dose group (DMT IR-RS, 4 g/kg BW); HG, high-dose group (DMT IR-RS, 8 g/kg BW); and PC, positive control group (glimepiride solution, 4 g/kg BW). In addition to the oral administration, all mice were fed with the normal columnar diet. Each result represents the mean values ± standard deviation ($n = 10$). [a]Represents statistical significance ($p < 0.01$) as compared to the MC.

After four weeks of gavage treatment, the fasting blood glucose levels in the LG, MG, MC and NC groups had increased to different levels, while the levels in the HG and PC groups were significantly lower than before. The fasting blood glucose levels in the high-dose group were lower than those of the other diabetic mice. These results demonstrated that RS can regulate blood sugar metabolism and lower the blood glucose levels of diabetic mice.

3.6. Effects of RS on organ indices

The organs of the mice from each group index are shown in Table 3. The spleen indices in the low-dose and middle-dose groups do not differ significantly from the model-dose group, while the high-dose group and positive control group differed significantly

Table 3
Organ indices in streptozotocin-induced diabetic mice ($n = 10$).

	Liver index (mg/g)	Kidney index (mg/g)	Spleen index (mg/g)
LG	61.2[a] ± 1.06	23.7[b] ± 1.01	5.40 ± 1.05
MG	58.4[a] ± 1.25	19.3[b] ± 0.681	5.50 ± 0.620
HG	56.0[a] ± 0.872	17.6[a] ± 1.40	4.47[b] ± 0.474
PC	54.3[a] ± 1.11	21.6[b] ± 2.17	3.90[b] ± 0.753
MC	66.1 ± 1.15	26.4 ± 1.24	7.43 ± 1.29
NC	53.9 ± 0.874	18.8 ± 0.620	3.33 ± 0.810

Organ index (mg/g) = liver, kidney and spleen weight/body weight of mice. NC, normal control group (distilled water, 4 g/kg BW); MC, model control group (distilled water, 4 g/kg BW); LG, low-dose group (2 g/kg BW); MG, middle-dose group (4 g/kg BW); HG, high-dose group (8 g/kg BW); and PC, positive control group (glimepiride solution, 4 g/kg BW). Each result represents the mean values ± standard deviation ($n = 10$).
[a] Indicates $p < 0.01$.
[b] Indicates $p < 0.05$ as compared to the MC.

Table 4
Effects of RS on the TC, TG and HDL (mmol/L) levels of the mice ($n = 10$).

	TC	TG	HDL
LG	5.18[b] ± 0.580	1.28 ± 0.190	1.18 ± 0.180
MG	4.90[b] ± 0.750	1.15 ± 0.130	1.55 ± 0.310
HG	3.24[a] ± 0.330	0.940[a] ± 0.120	1.62 ± 0.200
PC	3.78[a] ± 0.670	1.03[b] ± 0.180	1.65[b] ± 0.110
MC	6.55 ± 0.620	1.38 ± 0.120	1.26 ± 0.140
NC	2.65 ± 0.310	0.890 ± 0.0750	1.66 ± 0.0800

Each result represents the mean values ± standard deviation ($n = 10$). NC, normal control group (distilled water, 4 g/kg BW); MC, model control group (distilled water, 4 g/kg BW); LG, low-dose group (2 g/kg BW); MG, middle-dose group (4 g/kg BW); HG, high-dose group (8 g/kg BW); and PC, positive control group (glimepiride solution, 4 g/kg BW).

[a] Indicates $p < 0.01$.

[b] Indicates $p < 0.05$ as compared to the MC.

($p < 0.05$). The liver indices of the experimental mice in each group differed significantly ($p < 0.01$). The indices in the high-dose group and positive control group decreased by 15.3% and 17.8%, respectively, as compared to the model group.

The kidney indices of the 4 and 8 g/kg RS gavage groups were significantly lower than the model group. This effect was even higher than that observed in the positive control group. In summary, RS can effectively control diabetic liver, kidney and spleen hypertrophy in mice.

3.7. Comparison of serum lipid levels after treatment with RS

Diabetes is usually accompanied by the occurrence of symptoms of high cholesterol. Table 4 shows the serum lipid levels of mice. The serum TC and TG levels were significantly elevated, whereas the HDL-C levels were significantly decreased in the streptozotocin-induced diabetic control mice as compared to the normal controls. However, after 4 weeks of treatment with DMT IR-RS and glimepiride tablets, the alterations in serum lipids were partially attenuated, as evidenced by decreased serum TG and TC levels and by increased HDL-C concentrations in diabetic mice. The serum lipid levels of the PC group were similar to those of the NC group. There were no significant differences in HDL levels between the HG and MC groups. Compared to the MC group, the MG and HG groups had significantly lower ($p < 0.05$) TC or TG levels and similar HDL levels. This evidence indicates that RS can effectively control the symptoms of high cholesterol.

4. Conclusion

The present study demonstrated that dual modification treatment is an efficient method for the preparation of RS. Under the treatment conditions used in this study, the RS content was 47.0%. Structural modifications allowed our products to be strongly resistant to digestion. The DMT RS had beneficial health effects in diabetic mice. Significant correlations in diabetic mice have been established, including lower blood glucose levels in the HG than in the MC and higher body weights. Moreover, our products could reduce intestinal emptying time and positively impact intestinal health. Research is underway to investigate the efficacy of our products in intestinal metabolism.

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