

Research Article

Structure of Soluble Dietary Fiber from Fresh Rice at the Medium-Milk Stage and Improvement of Insulin Resistance in HepG2 Cells

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Fresh rice (*Oryza sativa* ssp. *japonica*) at the medium-milk stage is rich in soluble dietary fiber (SDF), leading to potential effects on type 2 diabetes (T2D). In this study, we analyzed each monosaccharide, the relative molecular weight, and the molecular linkages of fresh rice (grain, stem, husk, and leaf) SDF. The examined fresh rice leaf SDF consisted of 31.9% glucose, 35.7% galactose, 15.3% arabinose, 11.7% mannose, and a small amount of rhamnose and xylose. The molecular weight (M_w) was $232.5 \pm 19.1 \times 10^4$ u, and the radius of gyration (R_g) was 298.8 ± 25.4 nm. The glycosidic bonds consisted mainly of Araf and Glc/Gal and included 1,3-Araf, 1,5-Araf, and 3,5-Araf glycosidic bonds. The effect of fresh rice leaf SDF on insulin resistance (IR) in HepG2 cells showed that it could significantly enhance glucose consumption ($P < 0.05$). It also decreased the malondialdehyde (MDA) content ($P < 0.05$) and increased the total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities ($P < 0.05$) in a dose-dependent manner. Therefore, fresh rice leaf SDF might be a good dietary supplement for treating T2D.

1. Introduction

Rice (*Oryza sativa* ssp. *japonica*) is one of the staple foods in North China, occupying more than 4 million ha of farmed area and with 22 million tons produced in Heilongjiang Province [1]. During the ripening period, rice grains become hard, and the husk, leaves, and stems become completely yellow [2]. Here, fresh rice is referred to as rice harvested at the medium-milk stage between the maturation and wax maturation stages [3]. Studies have shown that the bioactive substances in rice gradually become more abundant from early maturation to the waxy stage [4]. The protein content in the medium-milk stage of fresh rice was the highest and was 1.5% higher than that in mature rice [3]. The lipid content in rice first increased and then decreased, and the lipids in the medium-milk stage of fresh rice had the highest anti-

oxidant activity [5]. Our previous studies showed that soluble dietary fiber (SDF) in fresh rice at the medium-milk stage had higher antioxidant activity, even though its structure remains unclear [6]. Therefore, research on the various parts of fresh rice and the study of nutrient and structural changes would be of great developmental value.

Due to the decrease in physical activity and traditional diet consumption, the number of patients with type 2 diabetes (T2D) has been predicted to increase to 642 million by 2040 [7]. HepG2 cells are a hepatoblastoma cell line with a phenotype similar to that of hepatocytes. They retain many biological characteristics of hepatocytes and are ideal cells for establishing an insulin resistance (IR) model [8]. Therefore, elaborating the potential effect of fresh rice SDF on T2D would be useful [9]. Reports have shown that IR HepG2 cells are a good model for studying the association

between T2D and SDF [10, 11]. However, the structure of fresh rice SDF and its effect on IR in HepG2 cells have not been reported.

In this study, the structure of SDF from various parts of fresh rice at the medium-milk stage was explored. Samples from the appropriate period were selected and further used to improve IR in HepG2 cells. The aim of the research was to provide theoretical and experimental support for the development of new types of rice products and add value to the utilization of rice.

2. Materials and Methods

2.1. Materials. Rice (*O. sativa* ssp. *japonica*, Longjing 31, main japonica rice variety in Heilongjiang Province) was obtained from Luobei Mingshan Farm (longitude: 131.113320, latitude: 47.626770; Hegang City, China). The total period of rice maturation was calculated to be 25 days (18/8/2017-11/9/2017). Rice samples from the premilk, medium-milk, postmilk, wax, and mature stages were isolated at five-day intervals. At the medium-milk stage (18/23/2017-8/27/2017), fresh rice (grain, stem, husk, and leaf) samples were stored at -80°C until use. HepG2 cells were provided by the Chinese Academy of Sciences Committee on Type Culture Collection Cell Bank (Shanghai, China). DMEM, penicillin, and streptomycin were obtained from Gibco Co., Ltd. (Grand Island, NY, USA). Cell Counting Kit-8 was purchased from Beyotime Biotechnology (CCK8, Shanghai, China). The glucose test kit, micromalondialdehyde (MDA) kit, total superoxide dismutase (T-SOD) kit, and glutathione peroxidase (GSH-Px) enzyme assay kit were obtained from the Nanjing Institute of Bioengineering (Nanjing, China).

2.2. Extraction and Purification of Fresh Rice SDF. Extraction and purification were performed according to a modified method [12]. One-gram samples of fresh rice (grain, stem, husk, and leaf) at the medium-milk stage were distilled in 30 mL of water and heated in a 470 W microwave for 30 min. A 0.2% high-temperature-resistant α -amylase (10000 U/mL) solution at pH 6.0 was added at 97°C for 32 min. After cooling, the pH was adjusted to 7.0, and 0.2% neutral protease (400 U/mL) was added at 60°C for 30 min. The pH was then adjusted to 4.5, and 0.2% glucosidase (3000 U/mL) was added at 60°C for 30 min. After denaturing the enzyme at 100°C for 5 min, the enzymatic hydrolysate was transferred to a $3\ \mu\text{m}$ membrane filtration unit. After the filtrate was concentrated, 4 volumes of 95% ethanol were added for alcohol precipitation for 24 h at room temperature. The precipitate was centrifuged at $3000 \times g$ for 5 min and dried at 95°C for 3 h. D101 macroporous resin (Haiguang Chemical Co., Ltd., Tianjin, China) was used to purify SDF. The specific conditions were as follows: the sample loading flow rate was 2 mL/min, and the mass concentration of the sample loading solution was 2 mg/mL at pH 10. The elution conditions were as follows: 70% ethanol was selected as the eluent, and the flow rate of the eluent was 1 mL/min.

2.3. Determination of the Monosaccharide Composition. The monosaccharide composition of fresh rice SDF was determined by gas chromatography-mass spectrometry (GC-MS, Agilent Technologies, Palo Alto, CA, USA) [13]. Briefly, one microliter of the pretreated sample standard was injected into a GC-MS instrument for analysis. The temperature was increased to 160°C – 210°C within 10 min at a flow rate of 1.2 mL/min and increased further to 240°C over 10 min. Then, the temperature was gradually increased to 250°C . An electron bombardment source was used for the analysis at an electron energy of 70 eV within 35–450 *m/z*.

2.4. Determination of the Relative Molecular Weight and Radius of Gyration. Based on the reported method, an SDF solution at a concentration of 2 mg/mL was prepared by dissolution at 60°C and filtering through a $3\ \mu\text{m}$ membrane [14]. The solution was examined by a high-performance size exclusion chromatography-multiangle laser light scattering-refractive index system (HPSEC-MALLS-RI, #321, Gilson, Middleton, WI, USA, conducted at Gangneung Wonji National University, Gangneung, Korea). The molecular mass (M_w) and radius of gyration (R_g) were determined using an online detection system. The mobile phase was 0.15 mol/L NaNO_3 and 0.02% NaN_3 , and the flow rate was 0.4 mL/min. The results were analyzed using ASTRA 6.1 software (Wyatt Technology Corporation, CA, USA).

2.5. Determination of the Molecular Linkages. Fresh rice SDF was first methylated and then treated with trifluoroacetic acid, hydrolyzing at 100°C for 6 h. This was followed by reduction and acetylation by sodium borohydride (NaBH_4) and anhydrous acetic acid, respectively. The sugar alcohol acetate derivatives were partially methylated. The linkage mode was determined by comparing the peak times in the gas chromatography spectrum with the ion peaks in the mass spectrum with those of SDF with various linkage modes [15].

2.6. Grouping and Effects of Fresh Rice Leaf SDF on IR in HepG2 Cells. An IR model of HepG2 cells was established for preliminary experiments [16]. After digestion, a total of 5.0×10^4 cells were inoculated into a 24-well plate, and modeling was performed when the cell culture reached approximately 85% confluence. The blank control group (A) was cultured in serum-free high-glucose DMEM. The IR model group (B) was grown in serum-free high-glucose DMEM supplemented with insulin at a concentration of 5×10^{-7} mol/L. The low-, medium-, and high-dose fresh rice leaf SDF groups with 50 $\mu\text{g}/\text{mL}$, 100 $\mu\text{g}/\text{mL}$, and 200 $\mu\text{g}/\text{mL}$ fresh rice leaf SDF in group B were grouped as groups C, D, and E, respectively. The metformin-positive control group (group B treated with 100 $\mu\text{g}/\text{mL}$ metformin) was named group F. Ten microliters of the supernatant was collected after 24 h. The glucose content in the culture medium was measured using a glucose assay kit at 505 nm and calculated using equation(1). Glucose inhibition was calculated

using equation(2).

$$\text{Glucose content (mmol} \cdot \text{L}^{-1}) = \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Standard}}} \times \text{standard solution concentration,} \quad (1)$$

$$\text{Glucose inhibition rate (\%)} = \frac{A - A_1}{A} \times 100, \quad (2)$$

where A is the glucose consumption in the blank control group and A_1 is the glucose consumption in the treatment group.

The CCK8 method was used to determine the viability of cells in the logarithmic growth phase [17]. After digestion, the density of the HepG2 cells was adjusted to 1.0×10^4 , and the cells were inoculated into 96-well culture plates. The total volume per well was $150 \mu\text{L}$, and each group consisted of 6 parallel wells. The cells were incubated for 24 h at 37°C in 5% CO_2 under saturated humidity. The cells were washed with PBS 2-3 times, and $15 \mu\text{L}$ of CCK8 solution was added to each well for 1-2 h of culture. The absorbance of the culture was tested at a wavelength of 450 nm with an ultraviolet spectrophotometer. Cellular activity was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD}_{\text{Test}} - \text{OD}_{\text{Blank}}}{\text{OD}_{\text{Comparison}} - \text{OD}_{\text{Blank}}} \times 100. \quad (3)$$

The effects of fresh rice SDF on glucose consumption, MDA content, T-SOD activity, and GSH-Px activity in IR HepG2 cells were determined following the kit instructions.

2.7. Data Processing. The structural tests were repeated in triplicate, and each cellular test was repeated six times. Data processing was performed mainly with IBM SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). Differences in the data were analyzed with one-way ANOVA and post hoc Duncan's test.

3. Results and Discussion

3.1. Monosaccharide Composition of Fresh Rice SDF. During the ripening period, the nutrients in the various parts of rice plants change continuously [6]. Fresh rice SDF is an interesting research material at the medium-milk stage. As shown in Table 1, the grain SDF was composed mainly of glucose (79.2%) and small amounts of galactose (8.9%), rhamnose (0.6%), arabinose (4.7%), mannose (5.1%), and xylose (1.5%). The monosaccharide compositions of the stem, leaf, and husk SDF were very similar, and they consisted mainly of glucose (32.1%, 31.9%, and 34.2%), galactose (30.9%, 35.7%, and 35.7%), arabinose (14.4%, 15.3%, and 7.1%), mannose (17.4%, 11.7%, and 18.2%), and small amounts of rhamnose and xylose. The levels of monosaccharides in fresh rice SDF were different because they originated from various tissues [18]. In barley soluble fiber, arabinose (BSF-60, 20.5%), glucose (BSF-20, 74.5%), and xylose (BSF-40, 42.6%) were the main monosaccharides analyzed [19]. Dif-

TABLE 1: Monosaccharide compositions in fresh rice SDF.

Type	Grain (%)	Stem (%)	Husk (%)	Leaf (%)
Rhamnose	0.6	1.9	3.4	2.2
Arabinose	4.7	14.4	7.1	15.3
Xylose	1.5	3.3	1.4	3.2
Mannose	5.1	17.4	18.2	11.7
Glucose	79.2	32.1	34.2	31.9
Galactose	8.9	30.9	35.7	35.7

ferences in monosaccharide compositions were observed among different varieties.

3.2. Relative Molecular Weight and Radius of Gyration. The two peaks of fresh rice stem SDF in the ranges of 38-46 min (peak I) and 46-49 min (peak II) are shown in Figure 1(a), and the 38-45 min (peak I) and 45-49 min (peak II) peaks of fresh rice grain SDF are shown in Figure 1(c). These results show that the polysaccharide compositions in the stem and grain of fresh rice are not uniform. Only one symmetrical peak was observed in the spectra of the fresh rice husk and leaf SDF (Figures 1(b) and 1(d)). The elution time was 46-49 min, indicating the presence of homogeneous polysaccharides. In addition, there were obvious peaks for the fresh rice stem, husk, grain, and leaf SDF, indicating that these samples might have contained a small amount of protein [15]. The largest M_w was observed for peak I of fresh rice grain SDF, with a value of $1239.8 \pm 112.2 \times 10^4$ u, and the smallest M_w was observed for peak I of fresh rice leaf SDF, with a value of $232.5 \pm 19.1 \times 10^4$ u (Table 2). R_g did not show a large difference in any part of the fresh rice SDF.

It is more difficult for SDF with a large M_w to enter an organism to exert its biological activity, while low M_w SDF is not active [20]. Generally, polysaccharide fragments with an M_w between 100 and 200 kDa have higher biological activity [21]. Therefore, fresh rice leaf SDF is more likely to be functional than other kinds of SDF.

3.3. Molecular Linkages in Fresh Rice SDF. The GC-MS results are shown in Table 3. Most of the main-chain glycosidic bonds in fresh rice grain SDF are glycosidic and 1,6-glycosidic bonds, accounting for 59.6% and 17.3% of bonds, respectively. The glycosidic bonds in fresh rice stem SDF were mainly Glc/Gal bonds, accounting for 28.7% of the total, as well as 1,4-Man/Glc/Gal, 1,6-Man/Glc/Gal, and 3,6-Man/Glc/Gal bonds, together accounting for 37.1% of the total. The glycosidic bonds in fresh rice husk SDF were mainly composed of Glc/Gal, as well as 1,6-Glc/Gal, 3,4-Glc/Gal, 4,6-Glc/Gal, and 3,6-Glc/Gal bonds, corresponding to a peak area of 69.9%. In contrast, the fresh rice leaf SDF glycosidic bonds consisted of Araf and Glc/Gal and included 1,3-Araf, 1,5-Araf, and 3,5-Araf glycosidic bonds, corresponding to a peak area of 20.9%. The other bonds in fresh rice leaf SDF were 1,4-Glc/Gal, 1,6-Glc/Gal, 3,4-Glc/Gal, 4,6-Glc/Gal, and 3,6-Glc/Gal glycosidic bonds, accounting for a peak area of 55.6%.

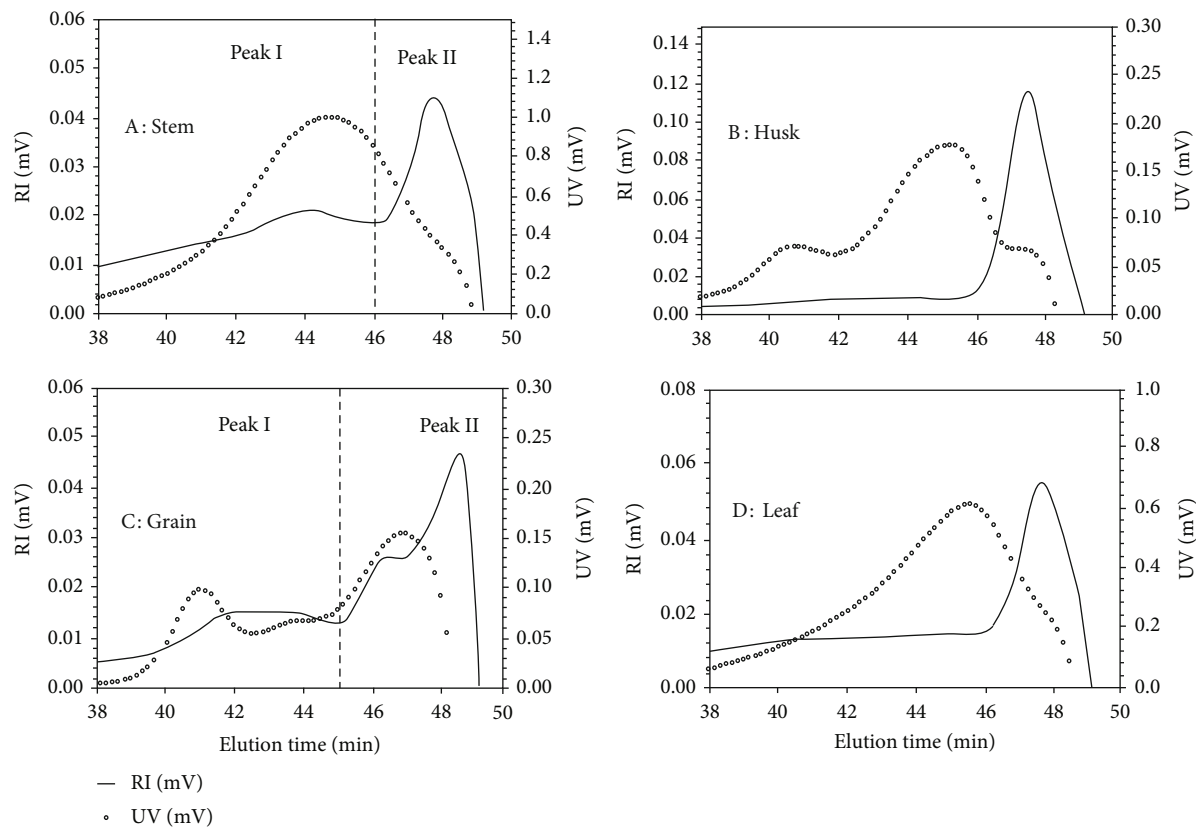


FIGURE 1: Refractive index and UV chromatography profiles of fresh rice SDF in various parts of rice.

TABLE 2: Molecular weight (M_w) and radius of gyration (R_g) in fresh rice SDF.

Sample	$M_w \times 10^4$ (u)		R_g (nm)	
	Peak I	Peak II	Peak I	Peak II
Stem	1239.8 ± 112.2	578.0 ± 9.2	309.4 ± 10.2	315.0 ± 11.2
Husk	274.1 ± 5.9	—	327.5 ± 13.1	—
Grain	1417.0 ± 158.5	539.8 ± 89.6	307.7 ± 14.6	297.7 ± 20.8
Leaf	232.5 ± 19.1	—	298.8 ± 25.4	—

Most polysaccharide materials with strong biological activity are linked by 1,3- and 1,6-glycosidic bonds, which exhibit a higher tumor inhibition rate than other bond types [22]. Other linkages, such as 1- and 4-linkages, are rarely active. In this study, fresh rice grain SDF had the lowest activity, and fresh rice leaf SDF had the highest activity.

3.4. Effect of Fresh Rice Leaf SDF on the Growth of IR and Glucose Consumption in HepG2 Cells. According to the above results, fresh rice leaf SDF is a potential candidate for further study. As shown in Table 4, the IR model group (B) showed 85.51% cell viability. The viability was not significantly different between the metformin-positive control group (F) and the blank control group (A), which indicated that metformin improved the IR in the HepG2 cells, as the cell viability in this group reached more than 99%. The low-dosage fresh rice leaf SDF group (C) and medium-

dosage fresh rice leaf SDF group (D) showed improved cell viability in comparison to the IR model group (B) ($P < 0.01$) after 24 h, and the cell viability was over 92.02%. The high-concentration fresh rice leaf SDF groups (E) significantly decreased cell viability ($P < 0.01$).

Glucose consumption by HepG2 cells is shown in Table 4. The IR model group (B) consumed the least glucose at 7.78 mmol/L. Glucose consumption by low- or high-dosage fresh rice leaf SDF (C or E) was greater than that in the IR model group (B) ($P < 0.01$), indicating that fresh rice leaf SDF could increase glucose consumption by HepG2 cells. The metformin-positive control group (F) also interfered with the inhibitory effect of insulin on the cellular uptake of glucose ($P < 0.01$). The effect of fresh rice leaf SDF in alleviating this effect of insulin was lower than that of the metformin-positive control group (F). The amount of glucose consumed by cells treated with different concentrations of SDF also differed. As the concentration of SDF increased, the amount of glucose consumed also increased. The group treated with a high-dosage of fresh rice leaf SDF (E) consumed 0.96 mmol/L more glucose than the IR model group (B). Furthermore, the group treated with 50 $\mu\text{g}/\text{mL}$ SDF (C) consumed 1.81 mmol/L more glucose than the group treated with 200 $\mu\text{g}/\text{mL}$ SDF (E).

It has been suggested that fresh rice leaf SDF adsorbs glucose, which reduces the concentration of glucose, promoting the uptake and metabolism of glucose by HepG2 cells and significantly improving their IR. SDF is also believed to improve the intestinal environment, increase

TABLE 3: Types of glycosidic bonds in fresh rice SDF at the medium-milk stage and corresponding peak areas.

Grain	Peak (%)	Stem	Peak (%)	Husk	Peak (%)	Leaf	Peak (%)
Araf-(1→	2.6	Araf-(1→	10.6	Araf-(1→	5.0	Araf-(1→	12.0
Rha-(1→	0.9	Rha-(1→	2.5	Rha-(1→	2.8	Rha-(1→	2.9
Glc-(1→	59.6	→3)-Araf-(1→	1.7	Xyl-(1→	1.2	→3)-Araf-(1→	1.8
Man-(1→	2.6	→5)-Araf-(1→	4.0	→3)-Araf-(1→	1.5	→5)-Araf-(1→	6.3
→2)-Glc-(1→	2.1	Glc/Gal-(1→	28.7	→5)-Araf-(1→	2.2	Glc/Gal-(1→	17.5
→3)-Glc-(1→	0.5	Man/Glc/Gal-(1→	5.4	Glc/Gal-(1→	33.6	Man/Glc/Gal-(1→	5.1
→4)-Glc-(1→	6.6	→3,5)-Araf-(1→	2.1	Man/Glc/Gal-(1→	6.7	→3,5)-Araf-(1→	0.8
→2)-Araf-(1→	2.2	→2)-Man/Glc/Gal-(1→	3.6	→2)-Man/Glc/Gal-(1→	3.1	→2)-Man/Glc/Gal-(1→	1.6
→6)-Glc-(1→	17.3	→3)-Man/Glc/Gal-(1→	1.3	→3)-Man/Glc/Gal-(1→	0.9	→3)-Man/Glc/Gal-(1→	2.1
→6)-man-(1→	1.6	→4)-Man/Glc/Gal-(1→	17.8	→4)-Glc/Gal-(1→	6.9	→4)-Glc/Gal-(1→	17.8
→2,5)-Araf-(1→	1.3	→3)-Man/Glc/Gal-(1→	3.0	→3)-Man/Glc/Gal-(1→	6.6	→3)-Man/Glc/Gal-(1→	4.8
→3,6)-Glc-(1→	2.6	→6)-Man/Glc/Gal-(1→	6.3	→6)-Glc/Gal-(1→	12.8	→6)-Glc/Gal-(1→	5.1
		→6)-Man/Glc/Gal-(1→	5.4	→6)-Glc/Gal-(1→	3.5	→6)-Glc/Gal-(1→	5.5
		→3,6)-Man/Glc/Gal-(1→	7.6	→3,4)-Glc/Gal-(1→	1.4	→3,4)-Glc/Gal-(1→	1.2
				→4,6)-Glc/Gal-(1→	6.4	→4,6)-Glc/Gal-(1→	3.2
				→3,6)-Glc/Gal-(1→	5.3	→3,6)-Glc/Gal-(1→	5.3

TABLE 4: The effect of fresh rice leaf SDF on the growth and glucose consumption of IR in HepG2 cells.

Type	Blank control group (A)	IR model group (B)	Low-dose SDF group (C)	Medium-dose SDF group (D)	High-dose SDF group (E)	Metformin positive control group (F)
Cell viability (%)	100.00 ± 3.24**	85.51 ± 0.93	92.71 ± 3.61**	92.02 ± 4.42**	77.48 ± 1.78**	99.02 ± 6.25**
Glucose consumption (mmol/L)	8.79 ± 0.43**	7.78 ± 0.27	10.55 ± 0.24**	8.12 ± 0.18*	8.74 ± 0.36**	8.86 ± 0.37**

Data were presented as means ± SE ($n = 6$). * $P < 0.05$, ** $P < 0.01$ vs. the IR model group (B).

the body's sensitivity to insulin, and improve methods of glucose consumption, resulting in an increased glucose consumption rate [23].

3.5. Effect of Fresh Rice Leaf SDF on the MDA Content and T-SOD and GSH-Px Activities of IR HepG2 Cells. Oxidative stress (OS) is positively correlated with IR, which plays a key role in the pathogenesis of T2D [24]. Antioxidant enzymes (such as SOD and GSH) and lipid peroxidation (such as MDA) can be used as OS evaluation markers [25]. The MDA content of the IR model group (B) was the highest, indicating that OS damage was the highest in this group among all the groups (Table 5). The MDA levels in the blank control group (A) and other groups of fresh rice leaf SDF and the metformin-positive control group (F) were significantly lower than that in the IR model group (B) ($P < 0.01$). These results suggest that both fresh rice leaf SDF and metformin could reduce the release of MDA in IR HepG2 cells. The MDA content in group E was 4.10 nmol/mg protein, which was lower than that in the IR model group (B)

($P < 0.01$). Thus, fresh rice leaf SDF could reduce the release of MDA and improve IR in HepG2 cells.

The T-SOD activity in the IR model group (B) was the lowest, and those in the fresh rice leaf SDF and metformin groups were significantly higher than that in the IR model group (B) (Table 5). Additionally, the T-SOD activity in group E was 38.99 ± 1.44 mg/protein, which was significantly higher than that in the IR model group (B) ($P < 0.01$). This result indicated that fresh rice leaf SDF could enhance the T-SOD activity of IR in HepG2 cells and significantly improve the degree of cell damage. These results were similar to those for SDF from whole-grain bean, whole soybean, whole-grain wheat, and whole-grain corn [26].

The GSH-Px activity was lowest in the IR model group (B), indicating that the POD activity was decreased in IR HepG2 cells. The GSH-Px activity in the fresh rice leaf SDF groups (C and D) ($P < 0.05$) and metformin-positive control group (F) ($P < 0.01$) was significantly higher than that in the IR model group (B) (Table 5). The GSH-Px activity in the group treated with 200 $\mu\text{g}/\text{mL}$ SDF (E) was 24.10

TABLE 5: MDA content and T-SOD and GSH activities of IR in HepG2 cells.

Type	Blank control group (A)	IR model group (B)	Low-dose SDF group (C)	Medium-dose SDF group (D)	High-dose SDF group (E)	Metformin positive control group (F)
MDA (nmol/mg protein)	3.79 ± 0.52**	5.82 ± 0.75	4.16 ± 0.54**	4.53 ± 0.65**	4.10 ± 0.54**	3.96 ± 0.82**
SOD (mg/protein)	57.01 ± 155**	32.13 ± 3.95	37.20 ± 1.46*	33.65 ± 0.73*	38.99 ± 1.44**	40.58 ± 1.33**
GSH (mg/protein)	36.32 ± 12.02*	24.43 ± 4.97	34.38 ± 7.75*	26.28 ± 3.39*	24.10 ± 10.12	35.36 ± 10.17*

Data were presented as means ± SE ($n = 6$). * $P < 0.05$, ** $P < 0.01$ vs. the IR model group (B).

± 10.12 mg/protein, which was not significantly different from that in the IR model group (B) ($P > 0.05$). Therefore, fresh rice leaf SDF at low concentrations could effectively improve IR in HepG2 cells and upregulate GSH-Px activity.

Animal experiments showed that dietary fiber from soybean okara could reduce insulin levels in T2D and induce IR, improving liver total antioxidant capacity (T-AOC) and SOD and GSH-Px activities and decreasing the MDA content [27]. Fresh rice leaf SDF could improve IR in HepG2 cells, providing a reference for the application of rice.

4. Conclusions

The amount of glucose among monosaccharides from the SDF of medium-milk-stage fresh rice grains was 79.2%, and the stem, leaf, and husk were mainly composed of glucose, galactose, and arabinose. Grain SDF is mainly linked through glucose and 1,6-glycosidic bonds, and stem, leaf, and husk SDF is mainly linked through glucose, 1,6-glucose, arabinose, and galactose. Additionally, fresh rice leaf SDF improved cell activity, significantly enhanced glucose consumption, reduced the MDA content, enhanced SOD activity, and increased GSH-Px activity, and these effects were enhanced with increasing concentrations. Therefore, this study demonstrated that fresh rice leaf SDF had a beneficial effect on IR in HepG2 cells. Although the mechanism was not clearly revealed, these results lay a foundation for future experiments to elucidate the specific mechanism.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Aiwu Zhang was responsible for the software (equal) and writing the original draft (equal). Ren Li was responsible for the data curation (equal) and validation (equal). Qi Wang was responsible for the methodology (equal). Guo-

liang Zhao was responsible for the investigation (equal). Linyan Zhang was responsible for the formal analysis (equal) and figure drawing (equal). Peng Jiang and Nian Liu were responsible for the resources (equal). Zhijiang Li was responsible for the conceptualization (equal) and funding acquisition (equal) and reviewed and edited the paper (equal). Aiwu Zhang and Ren Li contributed equally to the work and should be regarded as co-first authors.

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References

- [1] Z. J. Li, Y. H. Chen, D. J. Zhang, G. F. Zhang, and B. X. Lu, "Genetic diversity analysis and DNA fingerprinting of the main japonica rice varieties in Heilongjiang Province," *Quality Assurance and Safety of Crops & Foods*, vol. 11, no. 1, pp. 1–7, 2019.
- [2] Y. Matsue, K. Takasaki, and J. Abe, "Water management for improvement of rice yield, appearance quality and palatability with high temperature during ripening period," *Rice Science*, vol. 28, no. 4, pp. 409–416, 2021.
- [3] Y. Zhou, X. Y. Zhang, D. Li et al., "Optimization of protein extraction process and structural analysis of fresh rice," *Chinese Food Additives*, vol. 9, pp. 77–83, 2018.
- [4] Z. X. Wang, X. Y. Jiang, and Y. Han, "Comparative analysis of characteristics of main rice varieties in different periods in Liaoning Province," *Liaoning Agricultural Sciences*, vol. 3, pp. 20–22, 2015.
- [5] D. Li, C. Zhang, A. W. Zhang, L. L. Qian, and D. J. Zhang, "Changes of liposome and antioxidant activity in immature rice during seed development," *Journal of Food Science*, vol. 85, no. 1, pp. 86–95, 2020.
- [6] P. Y. Qu, D. Li, Z. J. Li, Y. Zhou, and D. J. Zhang, "Study on physicochemical characteristics and antioxidant activities of soluble dietary fiber in fresh rice," *Food Research and Development*, vol. 41, no. 1, pp. 36–42, 2020.
- [7] K. Ogurtsova, J. D. da Rocha Fernandes, Y. Huang et al., "IDF Diabetes Atlas: global estimates for the prevalence of diabetes

- for 2015 and 2040,” *Diabetes Research and Clinical Practice*, vol. 128, pp. 40–50, 2017.
- [8] A. M. Hossain, M. A. Brennan, X. Guo, X. A. Zeng, and C. S. Brennan, “Cellular biological activity and regulation of gene expression of antioxidant dietary fibre fraction isolated from blackcurrant incorporated in the wholemeal cereals cookies,” *Food Chemistry*, vol. 312, article 125829, 2020.
- [9] M. Ismaiel, H. Yang, and C. Min, “Dietary fiber role in type 2 diabetes prevention,” *British Food Journal*, vol. 118, no. 4, pp. 961–975, 2016.
- [10] M. Gurnell, D. B. Savage, V. K. K. Chatterjee, and S. O’Rahilly, “The metabolic syndrome: peroxisome proliferator-activated receptor γ and its therapeutic modulation,” *Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 6, pp. 2412–2421, 2003.
- [11] F. Fang, X. R. Wu, M. L. Luo, and H. Lv, “Establishment of IR model of HepG2 cells and its application in screening effective parts of mulberry leaves,” *Medical Herald*, vol. 31, no. 6, pp. 691–694, 2012.
- [12] X. J. Chen, X. F. Wu, S. T. Jiang, and X. J. Li, “Applying response surface methodology to optimize extraction of soluble dietary fiber from pear residue using hemicellulase,” *Food Science*, vol. 36, no. 6, pp. 18–23, 2015.
- [13] I. Ciucanu and F. Kerek, “A simple and rapid method for the permethylation of carbohydrates,” *Carbohydrate Research*, vol. 131, no. 2, pp. 209–217, 1984.
- [14] J. Xiao, R. A. Cao, J. Jia et al., “Purification of gentian polysaccharide by DEAE agarose gel and its molecular characteristics,” *Food Science*, vol. 37, no. 15, pp. 130–135, 2016.
- [15] R. A. Cao, X. L. Xu, J. W. Miao et al., “Structural characterization of gentian polysaccharide and its in vitro immune activity,” *Food Science*, vol. 38, no. 11, pp. 168–173, 2017.
- [16] X. Zheng, Y. Ke, A. Feng et al., “The mechanism by which amentoflavone improves insulin resistance in HepG2 cells,” *Molecules*, vol. 21, no. 5, article E624, p. 624, 2016.
- [17] J. Wang and J. H. Hu, *CCK8 method and MTT method to detect the inhibition rate of different concentrations of cisplatin on lung cancer cells A549*, Annual Meeting of Chinese Anatomical Society, Xining, China, 2015.
- [18] L. Jaime, E. Mollá, A. Fernández, M. A. Martín-Cabrejas, and R. M. Esteban, “Structural carbohydrate differences and potential source of dietary fiber of onion (*Allium cepa* L.) tissues,” *Journal of Agricultural and Food Chemistry*, vol. 50, no. 1, pp. 122–128, 2002.
- [19] Y. X. Wang, L. Y. Li, T. Zhang et al., “Fractionation, physico-chemical and structural characterization of polysaccharides from barley water-soluble fiber,” *Food Hydrocolloids*, vol. 113, article 106539, 2020.
- [20] S. Neupane, K. S. Bittkau, and S. Alban, “Size distribution and chain conformation of six different fucoidans using size-exclusion chromatography with multiple detection,” *Journal of Chromatography A*, vol. 1612, article 460658, 2020.
- [21] K. Li, Y. Cao, S. Jiao, G. Du, Y. Du, and X. Qin, “Structural characterization and immune activity screening of polysaccharides with different molecular weights from *Astragalus radix*,” *Frontiers in Pharmacology*, vol. 11, article 582091, 2020.
- [22] S. Demleitner, J. Kraus, and G. Franz, “Synthesis and antitumour activity of derivatives of curdlan and lichenan branched at C-6,” *Carbohydrate Research*, vol. 226, no. 2, pp. 239–246, 1992.
- [23] K. Ferrare, L. P. R. Bidel, A. Awwad et al., “Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract NCRAE of chicory *Cichorium intybus* L for an antidiabetic effect,” *Journal of Ethnopharmacology*, vol. 215, pp. 241–248, 2018.
- [24] L. Guan, H. Feng, D. Gong et al., “Genipin ameliorates age-related insulin resistance through inhibiting hepatic oxidative stress and mitochondrial dysfunction,” *Experimental Gerontology*, vol. 48, no. 12, pp. 1387–1394, 2013.
- [25] M. Amin, N. Rafiei, P. Poursafa et al., “Association of benzene exposure with insulin resistance, SOD, and MDA as markers of oxidative stress in children and adolescents,” *Environmental Science and Pollution Research*, vol. 25, pp. 34046–34052, 2018.
- [26] J. L. Guo, *Comparison of Biological Activities of Four Dietary Fibers and Improvement of IR Mechanism of HepG2 Cells Via P13K Pathway*, Yangzhou University, Yangzhou, China, 2014.
- [27] F. Li, *Intervention Effects of Okara, Okara Protein and Dietary Fiber on Type 2 Diabetes and the Mechanism*, Huazhong Agricultural University, Wuhan, China, 2015.