Antidiabetic Potential of Purple and Red Rice (Oryza sativa L.) Bran Extracts

Stephen M. Boue,*† Kim W. Daigle,† Ming-Hsuan Chen,‡ Heping Cao,† and Mark L. Heiman§

†Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70124, United States
‡Dale Bumpers National Rice Research Center, Agricultural Research Service, U.S. Department of Agriculture, 2890 Highway 130 East, Stuttgart, Arkansas 72160, United States
§Microbiome Therapeutics, 11001 120th Avenue, Broomfield, Colorado 80021, United States

ABSTRACT: Pigmented rice contains anthocyanins and proanthocyanidins that are concentrated in the bran layer. In this study, we determined the phenolic, flavonoid, anthocyanin, and proanthocyanidin content of five rice bran (1 brown, 2 red, and 2 purple) extracts. Each bran extract was evaluated for inhibitory effects on α-amylase and α-glucosidase activity, two key glucosidases required for starch digestion in humans. All purple and red bran extracts inhibited α-glucosidase activity, however only the red rice bran extracts inhibited α-amylase activity. Additionally, each bran extract was examined for their ability to stimulate glucose uptake in 3T3-L1 adipocytes, a key function in glucose homeostasis. Basal glucose uptake was increased between 2.3- and 2.7-fold by exposure to the red bran extracts, and between 1.9- and 3.1-fold by exposure to the purple bran extracts. In red rice bran, the highest enzyme inhibition and glucose uptake was observed with a proanthocyanidin-enriched fraction. Both IITA red bran and IAC purple bran increased expression of GLUT1 and GLUT4 mRNA, and genes encoding insulin-signaling pathway proteins.

KEYWORDS: brown rice bran, purple rice bran, red rice bran, adipocytes, glucose uptake, phenolics, anthocyanins, proanthocyanidins, α-glucosidase, α-amylase

Introduction

Diabetes mellitus is characterized by elevated levels of glucose in the blood. Diabetes is a growing health problem in the United States and has risen about 6-fold since 1950, now affecting approximately 29.1 million Americans and approximately 422 million people worldwide.1–3 About 79 million U.S. adults have prediabetes, which occurs when fasting blood glucose levels are 100–125 mg/dL. Prediabetics are at high risk of developing type II diabetes and other serious health problems. Without proper medical care, diabetes can lead to heart attack, stroke, blindness, kidney failure, and nerve damage.4,5 Type II diabetics have impaired sensitivity to secreted insulin from the pancreas and typically need more insulin to allow facilitated transport of glucose by glucose transporters. Glucose transporters (GLUTs) are membrane proteins and play a role in transporting glucose to most tissues. Basal uptake of glucose into tissues is mediated by GLUT1. Tissues such as skeletal muscle, liver, and adipose also contain GLUT4 for insulin-stimulated glucose uptake.4,5 Several pharmaceutical agents have been developed to improve glucose uptake, including pioglitazone and rosiglitazone.6,7 However, due to the many side effects caused by use of these medications,8 there is increased interest in natural hypoglycemic compounds or food ingredients containing bioactives that are considered to be less toxic.

Another method to manage diabetes is to inhibit the breakdown of starch to glucose during digestion by targeting the enzymes α-amylase and α-glucosidase. Pancreatic α-amylase is delivered into the intestinal lumen as a component of pancreatic juices, where it begins the chemical process of starch digestion. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose.9 α-Glucosidase is a membrane-bound enzyme located in the epithelium of the small intestine where it breaks down oligosaccharides and disaccharides to glucose. The inhibition of both α-amylase and α-glucosidase delays digestion and absorption of carbohydrates, and leads to suppression of postprandial hyperglycemia.10,11 Glucosidase inhibitors, like acarbose, are often prescribed to diabetics, but often produce diarrhea and other intestinal disturbances that limit their use for many diabetic patients.12 Due to these limitations, there has been an increase in research devoted to the location of food sources and supplements that may be more acceptable sources of glucosidase inhibitors.

Rice is one of the most important grains used in all parts of the world. Rice bran, a byproduct of rice milling, contains many bioactive compounds. These compounds include both lipophilic components (γ-oryzanol, tocotrienols, and tocopherols) and also phenolic compounds (ferulic acid, sinapic acid, and protocatechuic acid).13,14 Bioactives in rice bran have demonstrated beneficial health properties, including improved...
cardiovascular health,\(^{15}\) inhibition of cancer,\(^{16}\) and improvement of glucose homeostasis.\(^{17}\) Recently, research indicated that pigmented or colored rice bran contains both lipophilic and phenolic compounds with antioxidant activities.\(^{18,19}\) Further research demonstrated the ability of pigmented brans to inhibit cancer,\(^{16,20}\) inhibit \(\alpha\)-glucosidase activity,\(^{21}\) and prevent the development of fructose-induced insulin resistance in rats.\(^{22}\)

Although rice bran has shown potential to promote health, little information exists on the antidiabetic potential of rice bran, particularly pigmented rice brans. Here, we report the effects of brown, purple, and red rice bran extracts on the inhibition of two digestive enzymes (\(\alpha\)-amylase and \(\alpha\)-glucosidase). Additionally, this research compared the effect of brown rice bran and pigmented rice bran extracts on glucose uptake in 3T3-L1 adipocytes, and determined their effect on the gene expression of glucose transporters (GLUT1 and GLUT4) and components of the insulin-signaling transduction pathway. All five bran extracts were characterized for phenolic, flavonoid, anthocyanin, and proanthocyanidin content.

**MATERIALS AND METHODS**

**Chemicals and Reagents.** Folin–Ciocalteau reagent, cyanidin-3-glucoside, (+)-catechin, gallic acid, (+)-proanthocyanidin A2, trimethylsilyl acetonitrile, and ethanol were obtained from Sigma-Aldrich (St. Louis, MO). Proanthocyanidin A2 (proA2) was purchased from IndoFine Chemical Company (Hillsborough, N.J.)

**Sample Preparation.** Five rice cultivars (Oryza sativa L.) of different bran color (red, ITIA-119 (ITIA) and Wehani (Lundberg Farms, 2013); purple, IAC-600 (IAC) and Forbidden Rice (Lotus Farms, 2013); and light-brown Cecodrie (CCDR) varieties) were harvested in 2007. The IITA variety was harvested in 2009, and the IAC and CCDR varieties were harvested in 2007. ITIA from GRIN (PI 458466) (Germplasm Resources Information Network, http://www.ars-grin.gov) is a medium-grain rice originating from Nigeria. IAC is a short-grain rice developed by Candido Bastos of Agronomic Institute, Campinas, Brazil.

After removal of the hulls, whole grain rice was milled using a Satake Grain Testing Mill (model TM 05, Satake Engineering Co., Japan). During rice milling, rice bran is often contaminated with endosperm that would influence calculations of phytochemical concentrations (assays). Total starch content was determined (CCDR: 4.88%, IITA 18.1%, Wehani 10.4%, and Forbidden 9.91%) and used to correct all assay data. All bran samples were extracted three times with 70% ethanol at a 1:10 bran-to-solvent ratio for 2 h at room temperature (rt) with shaking (250 rpm). The supernatants of each extract were collected after centrifugation at 10000 \(\times\) g for 15 min, pooled, and then filtered through a 0.45 \(\mu\)m PVDF membrane. A portion of the filtrate was evaporated at reduced pressure and lyophilized. The dry extract was reconstituted in dimethyl sulfoxide at a concentration of 100 mg/mL for cell culture assays. A second portion of filtrate was evaporated and reconstituted with 70% ethanol.

**Preparation of ITIA Red Bran Fractions.** The method for producing red bran fractions was described by Chen et al.\(^{20}\) Briefly, bran (120 g) was extracted twice with hexane (1:8 bran to solvent ratio), followed by extraction twice with 70% ethanol for 2 h, centrifugation (7000 g, 4 °C, 15 min), pooling of supernatants, removal of ethanol at reduced pressure, and lyophilization. A 10 g bran equivalent extract was loaded onto a LH-20 cartridge and sequentially eluted with 120 mL of 20% methanol, 50% ethanol, and 70% acetonitrile to produce 3 fractions.

**Determination of Total Phenolics.** The Folin–Ciocalteau reagent method was used to determine the total phenolic content in the rice bran extracts.\(^{23}\) Sample extract (125 \(\mu\)L) was added to a test tube which contained 625 \(\mu\)L of Folin–Ciocalteau reagent (0.2 N), vortexed, and incubated at rt for 4 min. Next, 500 \(\mu\)L of 7.5% \(\text{Na}_2\text{CO}_3\) was added, vortexed, and incubated at rt for 30 min. Absorption at 760 nm was measured in a Shimadzu UV–vis spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample using a standard curve.

**Determination of Flavonoid and Proanthocyanidin Content.** Total flavonoid content of rice bran extracts was measured according to the method of Jia et al.\(^{24}\) Each sample (500 \(\mu\)L) was mixed with 5% \(\text{NaNO}_3\) (75 \(\mu\)L) and then left to stand for 5 min at rt. The mixture was sequentially mixed with 150 \(\mu\)L of 10% \(\text{AlCl}_3\), 500 \(\mu\)L of 1 M \(\text{NaOH}\), and 275 \(\mu\)L of distilled water. The absorbance was measured at 510 nm using a spectrophotometer. (+)-Catechin was used as a standard, and total flavonoid contents were expressed as catechin equivalents in milligrams per gram of sample. Proanthocyanidins were determined based on the method by Prior et al.\(^{25}\) Briefly, 70 \(\mu\)L of bran extract, control, and standard were placed into wells of a 96-well plate. Next, 210 \(\mu\)L of DMAC solution (0.05 g in 50 mL of acidified ethanol) was added. Acidified ethanol prepared using 12.5 mL of HCl, 12.5 mL of water, and 75 mL of ethanol. The absorbance was read at 640 nm every minute for 30 min.

**Determination of Anthocyanins.** Anthocyanins in the rice bran extracts were quantified using HPLC. The HPLC system consisted of a Phenomenex (Bellefonte, PA) Luna C18 column (3 mm id. × 25 cm), a Waters 2690 separation module, and a Waters 996 photodiode array detector. The mobile phase was a gradient mixture of A (6% formic acid in water) and B (acetonitrile), with the percentage of A ramped from 100% to 55% in 45 min with a constant flow rate of 0.8 mL/min. The concentration of each anthocyanin was calculated based on peak areas acquired at 520 nm and using a calibration curve (1–100 \(\mu\)g/mL) with cyanidin-3-glucoside.

**\(\alpha\)-Amylase and \(\alpha\)-Glucosidase Inhibition.** The methods for enzymatic inhibition assays were adapted from Apostolidis et al.\(^{26}\) For the \(\alpha\)-amylase assay, in a 96-well plate 50 \(\mu\)L of sample, buffer control, or positive control (2–500 \(\mu\)g/mL acarbose) was added to 100 \(\mu\)L of a 1 U/mL \(\alpha\)-amylase solution (from *Saccharomyces cerevisiae* in 0.1 M sodium phosphate buffer pH 6.9) and incubated for 10 min. A 50 \(\mu\)L aliquot of 5 mM p-nitrophenyl-\(\alpha\)-glucopyranoside solution (in 0.1 M sodium phosphate buffer pH 6.9) was added to each well and incubated for 5 min at 25 °C. Before and after incubation the absorbance was read at 405 nm by a BioRad 3550 microplate reader. Results are calculated as follows: % enzyme inhibition = [(Δabsorbance of control – Δabsorbance of extract)/Δabsorbance of control] × 100%. For the amylose assay, in a 20 mL glass tube 500 \(\mu\)L of sample, buffer control, or positive control (10–2000 \(\mu\)g/mL acarbose) was added to 500 \(\mu\)L of 13 U/mL \(\alpha\)-amylose solution (type VI-B from porcine pancreas in 0.02 M sodium phosphate buffer pH 6.9) and incubated for 10 min at 25 °C. Next, 500 \(\mu\)L of soluble starch solution (potato starch dissolved in sodium phosphate buffer pH 6.9) was added to each tube and incubated for 10 min at 25 °C. Last, 1 mL of dinitrosalicylic acid color reagent was added to each tube and placed in 100 °C water for 5 min. The mixture was diluted with 10 mL of distilled water, and the absorbance was read at 540 nm using a Shimadzu UV-1800 spectrophotometer. Results were calculated as follows: % enzyme inhibition = [(absorbance of control – absorbance of extract)/absorbance of control] × 100. All measurements were done in triplicate.

**3T3-L1 Mouse Adipocyte Cell Culture.** The method for cell culture was based on a previously reported method with some modifications.\(^{27}\) Murine preadipocytes (Zen-Bio Inc., Research Triangle Park, NC) were cultured using PM-1-L1 medium (Zen-Bio Inc.) in a humidified atmosphere (5% CO\(_2\), 95% air). After 3–4 days, when the cells reached confluence, the PM medium was replaced by the differentiation medium (DM-2-L1, Zen-Bio Inc.) for 3 days. The medium was then changed to AM-1-L1 medium (Zen-Bio Inc.), which was changed every 2–3 days during an additional 10 days of incubation.

**Glucose Uptake Assay.** The glucose uptake was carried out according to the method described previously.\(^{27}\) Adipocytes were grown in 24-well plates and used 10–11 days after initiation of differentiation. Adipocytes were rinsed in sterile, fresh KRH buffer, and
then preincubated for 24 h in KRH buffer. Adipocytes were incubated in KRH buffer containing bran extracts or DMSO control for the specified time period (18 h for glucose uptake; 8 and 12 h for RNA isolation). 10 μL of [1H]-2-deoxy-D-glucose (Vitrax, Placentia, CA) diluted to 0.01 μCi/μL with d-glucose (100 mM) was added to each well and incubated for 10 min in a 37 °C water bath. The supernatant was removed, and plates were rinsed rapidly three times with ice cold KRH. The final rinse was aspirated with care to not remove the cellular monolayer, and 500 μL of ice cold RIPA buffer (Sigma Chemical Co., St. Louis, MO) was added to lyse the cells. A 450 μL aliquot was added to 5 mL of scintillation fluid, mixed, and counted for 10 min in a liquid scintillation counter. Data are the average of 3 experiments that were normalized by calculating the percent cpm glucose uptake compared to basal cpm glucose uptake.

RNA Extraction and Quantitative RT-PCR. RNA isolation and RT-PCR were carried out according to previously reported methods. Additional sequences of the forward primer, reverse primer, and TaqMan probes for GLUT1, GLUT4, INSR, and IRS1 and the housekeeping gene ribosomal protein L32 (RPL32) (NM_172086) were described previously. Additional primer sets were as follows: Akt2, 5′-GAGATTGTGTCAGCTTGGAG-3′ and 5′-ACAGGCCCAAGTGTCGATCCT-3′; PI3K (catalytic, beta), 5′-AAAGACGGCACCCTGCAACTG-3′ and 5′-CGAGGACATAAGAGGCTACAC-3′. The PCR reactions were performed in a CFX96 Real-Time PCR Detection Systems (Bio-Rad, Hercules, CA). The ΔΔCt method of relative quantification was used to determine the fold change in the gene expression levels in the rice bran treated adipocytes compared to the DMSO-treated control. The threshold cycle (Ct) values of the target mRNAs were normalized to the Ct values of the internal control RPL32.

Statistical Analysis. All results are expressed as mean ± standard deviation (SD; n = 3). Statistical comparisons were performed using one-way ANOVA with Fisher (LSD) analysis of the differences between the categories with a confidence interval of 95%. A p-value below 0.05 was considered statistically significant. All statistical analyses were performed using XLSTAT 2007 for Excel (Addinsoft Software, Inc.).

RESULTS AND DISCUSSION

Characterization of Rice Bran Extracts. The concentrations of total phenolics, flavonoids, anthocyanins, and proanthocyanidins in five rice bran extracts (CCDR, brown; IAC and Forbidden, purple; IITA and Wehani, red) are shown in Table 1. The four pigmented brans had more than 4-fold higher phenolic content and more than 3-fold higher flavonoid content when compared to the brown bran (CCDR). The brown CCDR bran had the lowest phenolic content of 5.71 mg GAE/g and the lowest flavonoid content of 3.71 mg catechin equiv/g. The red Wehani bran displayed the highest phenolic content at 30.61 mg GAE/g, and the red IITA bran had the highest flavonoid content at 20.7 mg catechin equiv/g. Anthocyanins were detected at low levels in the red rice brans tested, and at high levels in the purple brans tested (12.0 mg/g for IAC and 5.36 mg/g for Forbidden).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total Phenolics (mg gallic acid equiv (GAE)/g)</th>
<th>Total Flavonoids (mg catechin equiv/g)</th>
<th>Total Anthocyanins (mg/g)</th>
<th>Total Proanthocyanidins (mg proA2 equiv/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocodrie</td>
<td>5.71 ± 0.58 a</td>
<td>3.71 ± 0.25 a</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>IITA</td>
<td>28.33 ± 0.17 c</td>
<td>20.7 ± 0.27 d</td>
<td>1.71 ± 0.55 a</td>
<td>22.1 ± 0.24 c</td>
</tr>
<tr>
<td>Wehani</td>
<td>30.61 ± 0.46 d</td>
<td>14.0 ± 0.54 c</td>
<td>0.42 ± 0.12 a</td>
<td>15.4 ± 0.29 b</td>
</tr>
<tr>
<td>IAC</td>
<td>27.41 ± 0.15 b</td>
<td>14.6 ± 0.29 c</td>
<td>12.0 ± 0.50 c</td>
<td>1.88 ± 0.06 a</td>
</tr>
<tr>
<td>Forbidden</td>
<td>27.65 ± 0.53 bc</td>
<td>12.8 ± 0.70 b</td>
<td>5.36 ± 0.29 b</td>
<td>1.98 ± 0.11 a</td>
</tr>
</tbody>
</table>

*Results are presented on a dry weight (DW) basis. The DW of rice bran was corrected for starch concentration. Values are mean ± SD (n = 3). ND, not detectable. Means with different letters (a to c) within the same column are significantly (p < 0.05) different. **proA2 = proanthocyanidin A2.

anthocyanin in the IAC and Forbidden bran extracts was cyanidin-3-glucoside (approximately 90–91.3%, data not shown). Both red rice brans, IITA and Wehani, contained high concentrations of proanthocyanidins at 22.1 and 15.4 mg proA2 equiv/g, respectively.

Rice is used primarily as a source of starch and nutrition for much of the world. The bran is typically milled and either discarded or used for animal feed. Pigmented rice brans offer the same lipophilic components as brown rice bran, but with the additional phenolic components that are often only found in fruits and some vegetables. Several researchers have identified anthocyanins in purple rice bran and proanthocyanidins in red rice bran. Our results in this study confirm high concentrations of anthocyanins in both IAC and Forbidden purple bran extracts, and proanthocyanidins in both IITA and Wehani red bran extracts. These phenolic compounds found in pigmented rice bran are the main difference in composition when compared to brown rice bran (CCDR).

α-Amylase Inhibition. The inhibitory effects of brown, red, and purple bran extracts on α-amylase activity are shown in Figure 1A. As expected, the positive control acarbose showed the highest inhibitory activity with 56.3% inhibition at 83 μg/mL and 91.4% inhibition at 2 mg/mL. The brown rice CCDR bran extract had no inhibitory effect on α-amylase activity. The two purple rice bran extracts, Forbidden and IAC, had only weak enzyme inhibition activity at the highest concentration (2 mg/mL) tested with inhibition at 6.03% and 11.6%, respectively. However, both red rice bran extracts, Wehani and IITA, displayed moderate levels of enzyme inhibition at 41.3% and 46.2% at 2 mg/mL. The activities of both red rice bran extracts were much lower than that of acarbose.

There is limited information available on the ability of pigmented rice bran extracts to inhibit α-amylase activity. Shimoda et al. showed that a purple rice bran extract inhibited α-amylase with an IC50 value of 135 μg/mL. Other plants containing phenolics have been shown to inhibit α-amylase, including grape and grape seed, blueberry, "rowanberry and raspberry," and several Brazilian fruit extracts. Some of the α-amylase inhibition of several extracts can be attributed to cyanidin-3-glucoside, which has been shown to inhibit α-amylase. However, Johnson et al. showed that a blueberry fraction containing proanthocyanidins has a lower IC50 value (IC50 = 31.4 μg/mL) for α-amylase when compared to the anthocyanin-enriched fraction (IC50 > 100 μg/mL). This would explain the higher inhibition activities observed for the two red brans (15.4–22.1 mg/g proanthocyanidins) when compared to the lower activity of the two purple brans (5.36–12.0 mg/g anthocyanins) in this study. Also, research has detailed the ability of proanthocyanidins (tannins) to inhibit α-
amylase activity, with the ability of more complex tannins, particularly high molecular weight ellagitannins from pomegranate, high molecular weight condensed tannins from cranberry, and a fraction containing proanthocyanidins from rowanberry, to bind with the α-amylase enzyme.32−34,37 α-Glucosidase Inhibition. The inhibitory effects of brown, red, and purple bran extracts on α-glucosidase activity are shown in Figure 1B. Acarbose was much less potent in inhibiting α-glucosidase when compared to its strong inhibitory effect on α-amylase. Acarbose at the highest dose tested (500 μg/mL) showed 37.1% inhibition of α-glucosidase (IC₅₀ > 500 μg/mL). The only rice bran extract to show lower inhibition compared to acarbose was the brown CCDR extract, which did not inhibit α-glucosidase (1.57% inhibition at 500 μg/mL). All four of the pigmented rice bran extracts displayed higher inhibition of α-glucosidase when compared to acarbose and the CCDR brown rice bran extract. The two purple rice bran extracts, IAC and Forbidden, showed inhibition of 73.4% and 84.4% at 500 μg/mL, respectively, with an IC₅₀ = 88.2 μg/mL for IAC and an IC₅₀ = 41.4 μg/mL for Forbidden. The two red rice bran extracts, Wehani and IITA, displayed higher enzyme inhibition values of 90.3 and 91.8% at 500 μg/mL, with an IC₅₀ = 45.2 μg/mL for Wehani and an IC₅₀ = 8.44 μg/mL for IITA.

Phenolic extracts from pigmented rice and rice bran have been shown to be effective inhibitors of α-glucosidase activity.21,30 In blueberry, a proanthocyanidin-enriched fraction (IC₅₀ = 25 μg/mL) had higher α-glucosidase inhibition when compared to the anthocyanin-enriched fraction (IC₅₀ > 100 μg/mL).33 Similar to α-amylase inhibition, our results point to the red brans containing proanthocyanidins as having higher inhibition activity. Further research by Shimoda et al. showed that a purple rice bran extract suppressed α-glucosidase activity between 300 and 1000 μg/mL (IC₅₀ = 409 μg/mL).30 In the study by Yao et al., one purple rice extract (IC₅₀ = 475 mg/mL) had higher α-glucosidase inhibitory activity when compared to a red rice extract (IC₅₀ > 1000 mg/mL).21 Our results differ from Yao et al. in that both red rice bran extracts had higher inhibition activity when compared to the two purple rice bran extracts, however both Wehani (red) and Forbidden (purple) were very similar in activity. A major difference in the two studies was that Yao extracted whole rice samples that included much starch, which reduced inhibition activity and anthocyanin content when compared to bran extracts. Also, varietal differences exist with some rice varieties containing less proanthocyanidins in red rice and less anthocyanins in purple rice.14,38

Increased Glucose Uptake in Adipocytes by Colored Bran Extracts. The potential of rice bran extracts to stimulate glucose uptake in 3T3-L1 adipocytes is shown in Figure 2. Insulin (0.2 nM) as a positive control increased basal glucose uptake 2.7-fold as shown in Figure 2. The brown rice bran extract (CCDR) did not significantly increase glucose uptake, however the four pigmented rice bran extracts significantly increase glucose uptake in 3T3-L1 adipocytes when compared to the untreated control. The symbols represent means ± SDs. All means with different letters are significantly (p < 0.05) different. 2DG = [³H]-2-deoxy-D-glucose.

Figure 1. Effect of light brown (CCDR), purple (IAC and Forbidden), and red (IITA and Wehani) bran extracts on inhibition of (A) α-amylase and (B) α-glucosidase activity. The symbols represent means ± SDs.
increased glucose uptake (Figure 2). The purple rice bran extracts, Forbidden and IAC, stimulated uptake (p < 0.05) from the basal glucose uptake rate by 1.9-fold and 3.1-fold at the highest dose (100 μg/mL), respectively. The Wehani red rice bran extract increased glucose uptake by 2.3-fold at 100 μg/mL. Maximal stimulation for the IITA red bran extract occurred at 50 μg/mL and was 2.7-fold greater than that measured for basal glucose uptake (p < 0.05).

The four pigmented rice bran extracts have varying concentrations of phenolics that include flavonoids, anthocyanins, and proanthocyanidins that appear to contribute to the stimulation of glucose uptake activity observed in this study. In research by Chen et al.,20 the IAC purple rice bran extract displayed high concentrations of ferulic acid, protocatechuic acid, vanillic acid, and total benzoic acids. Protocatechuic acid has demonstrated the ability to stimulate glucose uptake in omental adipocytes.39 Ferulic acid increased uptake of 2-deoxyglucose in L6 myotubes.40 Although ferulic acid is present in the IAC bran extract, it is also present in the CCDR bran extract,26 and the lack of glucose uptake by the CCDR brown rice bran extract indicates that it is not an active component responsible for IAC's activity. Anthocyanins were identified in the IAC (12.0 mg/g) and Forbidden (5.36 mg/g) purple brans with cyanidin-3-glucoside accounting for between 90 and 91.3% (data not shown) of this total anthocyanin amount. Several studies have demonstrated the ability of cyanidin-3-glucoside to ameliorate hyperglycemia and improve insulin sensitivity.41,42 These earlier studies indicated that high concentrations of cyanidin-3-glucoside and protocatechuic acid in purple bran are likely primary contributors to stimulating glucose uptake in adipocytes.

The IITA and Wehani red rice bran extracts in this study both contained high levels of proanthocyanidins. Proanthocyanidins are commonly found in many fruits including cranberry, grapes, cocoa, and apples25,43 and studies have reported many health-promoting activities.22-44 Red rice bran is an excellent source of proanthocyanidins and contained higher levels when compared to blueberries and broccoli.19 Grape seed derived proanthocyanidins given orally to streptozotocin-induced diabetic rats displayed an antihyperglycemic effect.45 The proanthocyanidin extract also stimulated glucose uptake in L6E9 myotubes and 3T3-L1 adipocytes in a dose-dependent manner, which demonstrated that proanthocyanidins have insulin-like effects both in vitro and in vivo.46 These previous studies point to the proanthocyanidins found in the IITA red rice bran as the components stimulating glucose uptake in this study.

### IITA Fractions Effect on Enzyme Inhibition and Glucose Uptake in Adipocytes

To further identify active components in red bran extracts contributing to enzyme inhibition, a second red IITA bran extract was fractionated through Sephadex. IITA was chosen due to its higher concentration of proanthocyanidins. Three fractions were obtained: 20% methanol, 50% ethanol, and 70% acetone. The inhibitory effects of the 20% methanol, 50% ethanol, and 70% acetone IITA fractions on α-amylase activity are shown in Figure 3A. The 20% methanol IITA fraction did not inhibit α-amylase activity with 1.06% inhibition at the highest dose tested (500 μg/mL). The 50% ethanol IITA fraction displayed moderate α-amylase inhibition with 27.4% inhibition at 500 μg/mL (IC50 > 500 μg/mL). However, the 70% acetone IITA fraction showed the highest α-amylase inhibition of 70.8% at 250 μg/mL with the lowest IC50 value (IC50 = 69.4 μg/mL).

**Figure 3.** Effect of three IITA red rice bran fractions (20% methanol, 50% ethanol, and 70% acetone) on inhibition of (A) α-amylase and (B) α-glucosidase activity.

Higher levels of enzyme inhibition activity for all 3 fractions were observed when examining the inhibition of α-glucosidase (Figure 3B). The 20% methanol IITA fraction had the lowest level of activity (IC50 = 162 μg/mL) with 80.9% inhibition at the highest concentration tested (500 μg/mL). The 50% ethanol fraction showed 89.3% α-glucosidase inhibition at 168 μg/mL (IC50 = 6.38 μg/mL). The highest activity (IC50 = 1.85 μg/mL) was observed with the 70% acetone fraction with 91.2% inhibition.

In this study, the inhibition data using both digestive enzymes indicate that the lowest activity was obtained with the 20% methanol fraction and the highest enzyme inhibition came from the 70% acetone fraction. Ming et al. determined
Table 2. Effect of Three Red Bran (IITA) LH-20 Sephadex Fractions on Glucose Uptake by 3T3-L1 Adipocytes: 20% Methanol, 50% Ethanol, and 70% Acetonea

<table>
<thead>
<tr>
<th>IITA red rice bran fractions</th>
<th>2DG uptake (% basal)</th>
<th>0 μg/mL</th>
<th>1 μg/mL</th>
<th>10 μg/mL</th>
<th>25 μg/mL</th>
<th>50 μg/mL</th>
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<tr>
<td>20% methanol</td>
<td></td>
<td>100.0 ± 16.4 a</td>
<td>123.9 ± 31.4 a</td>
<td>118.7 ± 20.1 a</td>
<td>135.7 ± 35.2 a</td>
<td>228.8 ± 58.0 b</td>
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<tr>
<td>50% ethanol</td>
<td></td>
<td>100.0 ± 8.7 a</td>
<td>110.7 ± 23.8 a</td>
<td>231.1 ± 44.5 b</td>
<td>334.4 ± 46.1 d</td>
<td>291.5 ± 48.7 c</td>
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<td>70% acetone</td>
<td></td>
<td>100.0 ± 13.8 b</td>
<td>193.1 ± 42.0 c</td>
<td>207.1 ± 43.1 c</td>
<td>65.7 ± 8.5 ab</td>
<td>47.8 ± 9.7 a</td>
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“Values represent means ± SDs. All means for glucose uptake with different letters within the same row are significantly (p < 0.05) different. 2DG = [3H]-2-deoxy-D-glucose.

Table 3. Effect of IAC and IITA Bran Extracts on mRNA Levels of Glucose Transporters and Insulin-Signaling Components in Mouse 3T3-L1 Adipocytesa

<table>
<thead>
<tr>
<th>mRNA</th>
<th>time (h)</th>
<th>control</th>
<th>IAC</th>
<th>IITA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>8</td>
<td>1.00 ± 0.03 a</td>
<td>1.03 ± 0.07 a</td>
<td>12.1 ± 0.26 b</td>
</tr>
<tr>
<td>GLUT4</td>
<td>8</td>
<td>1.00 ± 0.08 a</td>
<td>3.76 ± 0.09 b</td>
<td>17.2 ± 0.06 c</td>
</tr>
<tr>
<td>Akt2</td>
<td>8</td>
<td>1.00 ± 0.06 a</td>
<td>2.98 ± 0.23 b</td>
<td>8.54 ± 0.18 c</td>
</tr>
<tr>
<td>PI3K</td>
<td>8</td>
<td>1.00 ± 0.04 a</td>
<td>1.10 ± 0.05 a</td>
<td>4.10 ± 0.25 b</td>
</tr>
<tr>
<td>INSR</td>
<td>8</td>
<td>1.00 ± 0.04 a</td>
<td>1.10 ± 0.05 a</td>
<td>4.10 ± 0.25 b</td>
</tr>
<tr>
<td>IRS1</td>
<td>8</td>
<td>1.00 ± 0.03 a</td>
<td>1.10 ± 0.05 a</td>
<td>4.10 ± 0.25 b</td>
</tr>
</tbody>
</table>

“Values are mean ± SD (n = 3). ND, not detectable. Means with different letters (a to c) within the same row are significantly (p < 0.05) different.

GLUT1 expression 3.76-fold and GLUT4 expression 2.98-fold. The IITA bran significantly increased GLUT1 gene expression at both the 8 and 12 h time points with increased GLUT1 expression of 12.1-fold and 17.2-fold, respectively. GLUT4 gene expression was also significantly increased 5.35-fold at 8 h, and 8.54-fold at 12 h.

Glucose uptake by adipocytes is dependent on the activity of glucose transporters (GLUTs). Both GLUT1 and GLUT4 genes are expressed as major GLUT mRNAs in differentiated 3T3-L1 adipocytes. In our study, in agreement with the glucose uptake results, the expression of both GLUT1 and GLUT4 was significantly upregulated by both pigmented bran extracts in differentiated 3T3-L1 cells. Both IAC and IITA bran extracts significantly increased the gene expression levels of both GLUT1 and GLUT4. These findings offer, for the first time, an insight into the mechanism for pigmented rice bran mediated glucose uptake by adipocytes. Pigmented rice bran extracts may act in concert with insulin producing increased GLUT4 mRNA, or independently of the hormone, by stimulating production of GLUT1 mRNA that is necessary for the biosynthesis of GLUT proteins to promote transfer of glucose into adipocytes. Although GLUT proteins were not measured in this study, it is likely that pigmented rice bran
induced GLUT4 expression could result in increases of the protein in adipocytes since several studies have detailed a positive correlation between GLUT4 mRNA and protein levels. The increased GLUT1 and GLUT4 mRNA levels with two pigmented rice bran extracts reported here suggest a positive effect of their phenolic compounds on long-term regulation of glucose transport.

**IITA Red and IAC Purple Bran Effects on Insulin-Signaling Component mRNA Levels.** We expanded PCR assays for another 4 genes coding for key components in the insulin-signaling pathway. After 8 h treatment, both IITA and IAC significantly increased Akt2 mRNA expression 4.59-fold and 2.29-fold, respectively, as shown in Table 3. IITA also increased Akt2 mRNA significantly (4.10-fold) after 12 h treatment (Table 3). PI3K mRNA was significantly increased 1.44-fold only after 8 h treatment with the IAC bran extract. INSR mRNA was significantly increased 2.22-fold after IAC treatment for 8 h and 1.45-fold after IITA treatment for 12 h. IRS1 was increased 2.73-fold after IAC treatment for 8 h and 3.26-fold after IITA treatment for 12 h.

Several molecular mechanisms explain how the stimulation of glucose uptake occurs, particularly related to increased expression of GLUT4 mRNA. Akt has emerged as a crucial transducer of the insulin-signaling cascade leading to GLUT4 translocation and glucose uptake. Of the three Akt isoforms, Akt2 has been strongly linked to GLUT4 translocation, and several studies have determined its importance related to insulin resistance and diabetes.

In our study, both IITA red rice bran and IAC purple rice bran stimulated Akt2 mRNA expression. Additionally, IRS1 plays a key role in insulin-stimulated glucose uptake, and our results indicated increased mRNA expression after 8 h treatment with IAC bran and 12 h treatment with IITA bran. Further, PI3K is the most widely accepted signaling enzyme necessary for insulin-stimulated glucose transport, and IAC bran at 8 h increased PI3K gene expression. Gene expression of the insulin receptor (INSR) was stimulated with the IAC and IITA bran extracts. The additional results presented here support the hypothesis that purple and red rice bran have insulin-like activities on regulation of glucose uptake and gene expression in mouse adipocytes.

In conclusion, all four pigmented rice bran extracts tested in this study showed activity at inhibiting α-amylase activity. Only red bran extracts were effective at inhibition of α-amylase activity. The IITA bran fraction (70% acetone) containing higher molecular weight proanthocyanidins (degree of polymerization >10) was more active at inhibiting α-amylase, and two fractions (50% ethanol and 70% acetone) containing low and high molecular weight proanthocyanidins inhibited α-glucosidase activity. Additionally, all four of the pigmented bran extracts significantly stimulated glucose uptake in adipocytes. Further testing of IITA bran fractions showed that both fractions containing proanthocyanidins had higher levels of increased glucose uptake. Additionally, the results presented here support the hypothesis that both purple and red bran extracts have insulin-like and insulin-independent effects on the regulation of gene expression in mouse adipocytes. The insulin-like effects of both colored rice brans on gene expression included the increased expression of specific insulin-signaling pathway genes. The effects of both colored rice brans that are insulin-independent include mainly increased GLUT1 gene expression. Our results indicate that colored rice brans have the potential to manage diabetes by both removal of blood glucose from the bloodstream into adipocytes and inhibition of starch conversion to glucose in the intestinal tract.


