Supplementary data

Modulation effect of black rice dietary fiber on the metabolism and fermentation of cyanidin-3-glucoside in an *in vitro* human colonic model

Shuai Zhang[a, b], Mei Deng[a], Ruifen Zhang[a], Xuchao Jia[a], Fei Huang[a], Dong Zhao[a], Lihong Dong[a], Jianwei Chi[a], Zhida Sun[b], Qin Ma*[a] and Mingwei Zhang*[a, b]

Supplementary Materials and methods

Preparation of Cy3G and purity analyses

Black rice of the indica cultivar Chifeng was purchased from Shandong Helaixiang Food Co., Ltd. (Shandong, China) and was milled to obtain rice bran using a PCM-20A Rice Milling Machine (Heilongjiang Province Wanmu Technology Co. Ltd., Heilongjiang, China). The rice bran was defatted by extraction with low-temperature continuous phase transition extraction technology (*n*-butane as extraction solvent) to get defatted rice bran. Defatted rice bran was ground to a fine powder to enable passage completely through an 80-mesh sieve on a Cyclone Sample Mill (UDY Corp., Fort Collins, CO) and then mixed with acidified ethanol (ethanol absolute and 1 M HCl 85:15, v/v) with 1:20 (w/v) and extracted at 50 °C for 2 h in a TE-MT-50 Ultrasonic Vacuum Extraction Tank (Shanghai Triowin Intelligent Machinery Co. Ltd., Shanghai, China). The supernatant was separated by centrifugation at 4 °C (4000 × *g*, 10 min), and the extraction procedure was repeated twice. The three supernatants were pooled and evaporated to dryness using a TW-RF10 Climbing Film Evaporator (Shanghai Triowin Intelligent Machinery Co. Ltd., Shanghai, China) to obtain crude extracts of Cy3G. Then the Cy3G extracts were successively purified by HPD826 macroporous resin (Cangzhou Bon Adsorber Technology Co. Ltd., Cangzhou, China) and LC3000 medium pressure liquid chromatography (Beijing ChuangXin Tongheng Sci. & Tech. Co., China) according to a previously published method.[1]

The obtained Cy3G was lyophilized and then characterized by HPLC-2030 Plus system (Shimadzu, Kyoto, Japan) equipped with an SPD-M20A photodiode (PDA) detector at the

wavelength of 520 nm, using a 250 × 4.6 mm, 5 μm Agilent Zorbox SB-C18 column (Palo Alto, CA, USA). The mobile phase consisted of solution A (1% formic acid in water, v/v) and solution B (acetonitrile), with the following gradient program: 0–2 min, solution B 8–12%; 2–5 min, solution B 12–18%; 5–10 min, solution B 18–20%; 10–12 min, solution B 20–25%; 12–20 min, solution B 25–30%; 20–23 min, solution B 30–45%; 23–25 min, solution B 45–80%; 25–27 min, solution B 80–8%; 27–35 min, solution B 8–8%, flow rate was 0.8 mL/min, injection volume was 10 μL. The purity of Cy3G was higher than 95% after calculated using a standard curve.

Preparation of IDF and purity analyses

The preparation of IDF was performed according to previously reported methods with modifications.[2] Briefly, IDF was extracted from the residue from the Cy3G extraction. The residue mixed with water (1:10, w/v) was first gelatinized at 95 °C for 10 min in a water bath, and then subjected to sequential enzymatic digestion with 0.3% *α*-amylase (pH 6.0, 95 °C for 30 min), 0.5% protease (pH 7.5, 60 °C for 60 min) and 0.2% amyloglucosidase (pH 4.5, 60 °C for 30 min). Then the mixture was centrifuged at 4000 × *g* for 10 min. The precipitate was washed with 80% ethanol and then hydrolyzed with 2 M NaOH (1:10, w/v) under N_2 atmosphere at 25 °C for 4 h in an Incubator Shaker. After hydrolyzation, the mixture was adjusted to neutral with 6 M HCl and centrifuged at 4000 × *g* for 10 min. The precipitate was washed successively with 80% ethanol and distilled water, then dried at 40 °C in a vacuum oven to obtain the IDF.

The dried IDF was ground to a fine powder to enable passage completely through an 80-mesh sieve and stored at −20 °C until use. The moisture, protein, fat, starch, ash and crude fiber content of IDF were determined according to AOAC Methods (2005) (AOAC 934.01, AOAC 960.52 using a conversion factor of 5.95, AOAC 948.22, AOAC 978.10, AOAC 942.05 and AOAC 985.29, respectively). The IDF contained 7.78% moisture, 9.51% protein, trace fat, 1.97% starch, 4.28% ash and 78.66% crude fiber.

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Figure captions

Fig. S1 Effects of IDF from black rice on the metabolism of Cy3G in the presence (Blank, Cy3G, IDF, and Cy3G+IDF groups) or absence (Cy3G unfermented, IDF unfermented, and Cy3G+IDF unfermented groups) of gut microbiota during *in vitro* colonic fermentation for 24 h. (A) Cyanidin-3-O-glucoside, (B) cyanidin, (C) protocatechuic acid, (D) hippuric acid, (E) vanillic acid, (F) p-hydroxybenzoic acid, (G) ferulic acid (H) caffeic acid. Data show mean ± standard deviation (n = 3).

Fig. S2 Effects of IDF from black rice on total phenolic content and antioxidant properties of fermentation broth of Cy3G in the presence (Blank, Cy3G, IDF, and Cy3G+IDF groups) or absence (Cy3G unfermented, IDF unfermented, and Cy3G+IDF unfermented groups) of gut microbiota during *in vitro* colonic fermentation for 24 h. (A) Total phenolic content, (B) FRAP, (C) ORAC, (D) PSC. Bars with no letters in common are significantly different ($p < 0.05$). Data show mean ± standard deviation (n = 3).

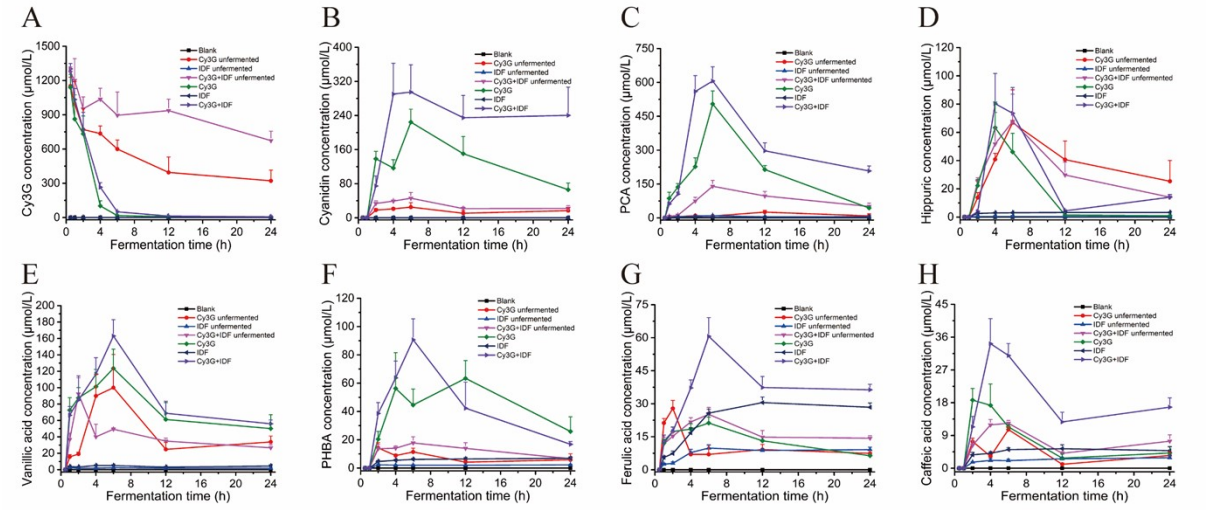

Fig S1

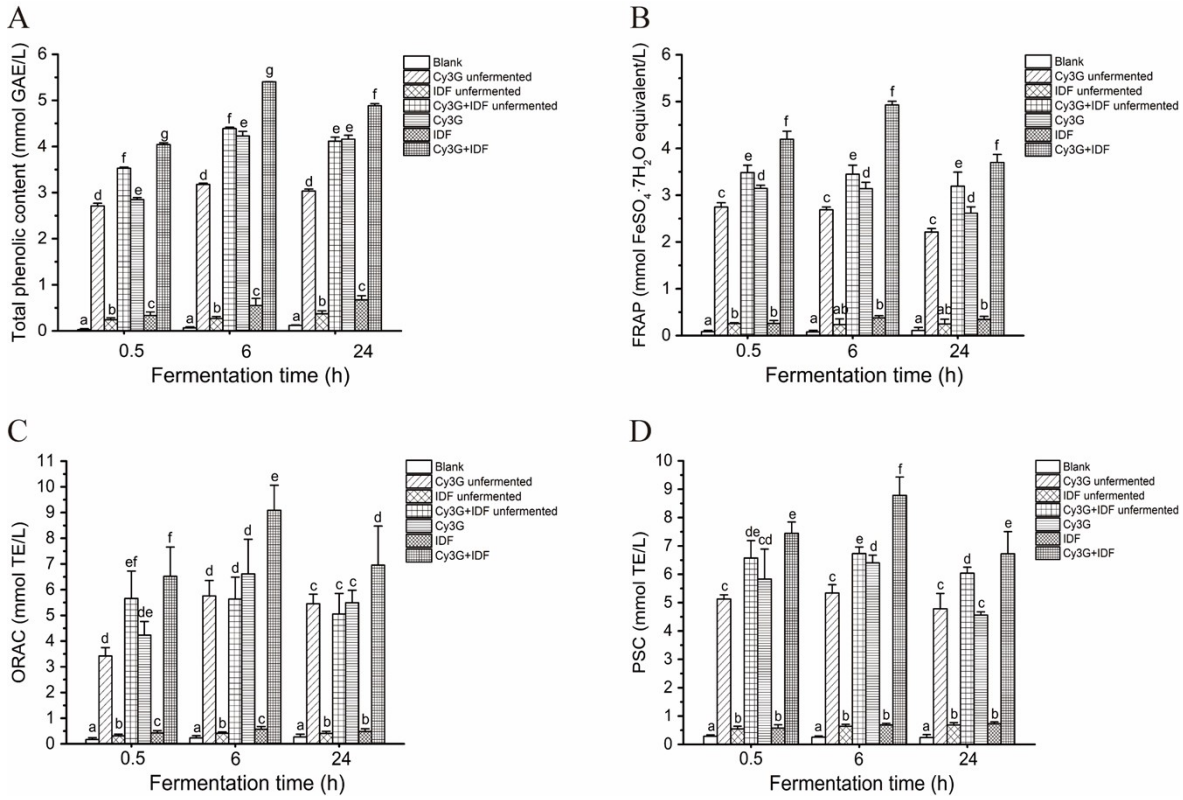

Fig S2

Reference

1. X. Jiang, H. Guo, T. Shen, X. Tang, Y. Yang and W. Ling, Cyanidin-3-O-beta-glucoside Purified from Black Rice Protects Mice against Hepatic Fibrosis Induced by Carbon Tetrachloride via Inhibiting Hepatic Stellate Cell Activation, *J. Agric. Food Chem.*, 2015, **63**, 6221-6230.
2. X. Zhang, M. Zhang, L. Dong, X. Jia, L. Liu, Y. Ma, F. Huang and R. Zhang, Phytochemical Profile, Bioactivity, and Prebiotic Potential of Bound Phenolics Released from Rice Bran Dietary Fiber during in Vitro Gastrointestinal Digestion and Colonic Fermentation, *J. Agric. Food Chem.*, 2019, **67**, 12796-12805.