

ABSTRACT

Non-digestible oligosaccharides (NDOs) have gained attention for their potential to modulate gut microbiota and enhance metabolic health. Pigmented cereals such as black rice (*Oryza sativa* L.) are an intriguing and unexplored source of functional oligosaccharides. In the present study, NDOs from Manipuri black rice were isolated, purified and structurally characterized, followed by *in vitro* and *in vivo* analysis of their prebiotic and anti-diabetic potential. Oligosaccharides were extracted under optimal conditions (80 % aqueous ethanol (v/v) at a sample-to-solvent ratio of 1:5 and a temperature of 60 °C for 60 min), providing a crude oligosaccharide enriched extract corresponding to 4.95 ± 0.33 % (w/w) relative to flour mass. A further purification by charcoal-celite adsorption chromatography and dialysis (500 Da MWCO) resulted in black rice oligosaccharides (BO) fraction (2.87 % (w/w)). MALDI-TOF MS revealed sodium adduct ions ranging from m/z ~504 to 1330, confirming enrichment of the DP3–DP8 oligosaccharides. Subsequent Bio-Gel P-2 gel filtration yielded a predominant purified fraction (BO1) with m/z 527.89 and MS/MS fragmentation consistent with hexose trisaccharide. FTIR, monosaccharide profiling and methylation analysis suggested a raffinose family oligosaccharide motif, while 1D and 2D NMR established BO1 as raffinose with α -Galp-(1→6)-GlcP and GlcP-(1→2)- β -Fruf linkages. For recovery enhancement, pre-extraction treatments including microwave-assisted (M), ultrasonic (U) and enzymatic extraction (E) were carried out along with the combined MUE process that exhibited highest yield (8.60 g/100 g) compared to untreated control. Fractionation steps resulted in water extractable and alkali soluble non-starch polysaccharides that exhibited minimal depolymerization under physical treatments (<1 %), while enzymatic processing achieved ~40–41% conversion, increasing to 46.18 % with ultrasound assistance, supporting scalable oligosaccharide production. *In vitro* assessment indicated that BO resisted simulated salivary, gastric, and pancreatic digestion, thereby meeting the non-digestible requirement for prebiotic selection. BO selectively enhanced the growth of *Lactobacillus* spp. and increased short-chain fatty acid (SCFA), mainly acetate, production. BO significantly promoted both probiotic biofilm formation (~3.9-fold) and adhesion to intestinal epithelial cells (IEC-6) with a faster rate of epithelial wound healing. Immunoregulatory assays showed that LPS-induced nitric oxide production was suppressed and stimulation of phagocytosis activity in RAW264.7 macrophages. BO promoted glucose consumption (~1.8-fold) in insulin-resistant HepG2 cells, indicating its application in metabolic health. *In vivo* evaluation using the streptozotocin-induced diabetic Balb/c mouse model, showed that BO had a low glycemic index (GI 20.9) and significantly ameliorated glycemic control, glucose tolerance, and insulin sensitivity. BO supplementation also restored lipid and organ-function markers as well as insulin and GLP-1 levels suggesting a possible interconnection between BO-supplementation and metabolic control. Taken together, BO possesses multivalent prebiotic effects and has the capacity to enhance probiotic colonization, epithelial repair, immune homeostasis and glucose tolerance suggesting its potential use in gut-directed functional foods and nutraceuticals for dietary management of lifestyle-related disorders such as diabetes.

Keywords: Black rice, Oligosaccharides, Prebiotic, SCFA, Functional food, Antidiabetic.