ISOLATION, PURIFICATION AND CHARACTERIZATION OF NON-DIGESTIBLE OLIGOSACCHARIDES (NDOs) FROM PEARL MILLET (*Pennisetum glaucum*) GRAIN

ABSTRACT

Pearl millet being a grain crop is a potential economically viable source to develop new class of prebiotics in the form of its oligosaccharides. In the present study, oligosaccharides were extracted at optimised process parameters and further purified through charcoal column, dialysis and gel filtration chromatography to obtain 6 of the purified oligosaccharide fractions having molecular weight 504, 667, 829, 991, 1153, 1316 Da. Glucose, fructose and galactose were the compositional monomers established from GC-MS/MS analysis. The 1D/2D NMR analysis confirmed these oligosaccharides to be Raffinose Family Oligosaccharides (RFOs).

Additionally, water soluble and alkali soluble polysaccharides were extracted separately. These were purified and further structurally characterised using methods such as silylation, methylation and NMR analysis. Thus, these polysaccharides now can be cleaved further for the large scale production of non-digestible oligosaccharides using various chemical and enzymatic methods.

The non-digestibility and the capability to selectively promote the growth of probiotics fulfil the criteria of prebiotic molecules. A positive prebiotic score, enhanced biofilm formation and capability of producing SCFAs (i.e. acetate, propionate and butyrate) by different strains of *Lactobacillus* sp. in presence of purified pearl millet oligosaccharides (PMO) hints towards their prebiotic potential. Furthermore, it increased the glucose consumption by 1.8 fold ($p \leq 0.05$) at 1000 μg/ml, in HepG2 cells. In another study, the oligosaccharides exhibited immunostimulating properties on RAW 264.7 macrophage cell line through NO production and phagocytosis assay.

In in vivo study, a daily intake of PMO (100 and 800 mg/kg body weight) showed reduction in blood glucose level, and biochemical parameters (triglycerides, LDL, urea, creatinine, SGOT, SGPT) in serum, compared to untreated group. Down-regulation of pro-inflammatory cytokines IL-6, TNF-α hints towards its anti-diabetic potential.

Increased accumulation of SCFAs in the caecal and fecal content of T2D bearing mice upon oligosaccharide administration resulted in modulation of GLP-1 and insulin concentration, in gut lumen and GLUT-4 expression in the epidydemal fat tissue as evident from ELISA and Western blot based studies respectively.

Keywords: Pearl millet, Oligosaccharides, Prebiotic, SCFA, Anti-diabetic properties