

Chapter 4

To study the *in vitro* antidiabetic activity of modified dietary fiber

4.1 Introduction

The digestive system is in charge of breaking down and absorbing the nutrients in our food (Sensoy et al., 2021). Microbes that are naturally present in the intestinal lumen aid in this process. The colon and small intestine are made up of many cell types that facilitate the passage of nutrients to the rest of the body via a single-layered epithelium that confronts luminal material and secretes digestive and absorption-related enzymes (Motta et al., 2021). Because the epithelial layer is always in close contact with luminal microorganisms, interactions with intestinal epithelial cells are essential to the long-term upkeep of the colon and small intestine. Studying the interactions between microorganisms and non-transformed primary human epithelial cells has proven to be extremely challenging (Ranganathan et al., 2020). When cultivated in a transwell system, the Caco-2 cell line creates a confluent and impermeable epithelial layer that has been widely used as a model of the intestinal epithelium, demonstrating multilineage differentiation (Schoultz and Keita, 2020). It is unknown, though, how effectively Caco-2 cells mimic a normal epithelium because they are an immortalized cancer cell line with abnormalities in important regulatory genes. The intricate physiological barrier that divides the intestinal lumen from the underlying mucosal tissue is the result of the evolution of intestinal epithelial cells. This barrier permits regulated access to antigens and the peaceful coexistence of the commensal resident microbiota with the human host while protecting against invading pathobionts (Fedi et al., 2021). This significance is made possible by some functional aspects of the epithelium, including the secretion of a mucus layer and the lifelong regeneration of several epithelial cell types from a stem cell compartment, which enables prompt repair of barrier deficiencies.

Human cell lines are widely used for carefully evaluating the harmful characteristics of both novel and well-known toxic compounds. The chemical, pharmaceutical, medical, and cosmetic sectors value these experiments. Since cell line-based assays are inexpensive, highly reproducible, and have a bioethical basis, they are generally chosen over animal model-based assays (Pamies et al., 2022). The liver, an essential organ for maintaining chemical homeostasis and a player in protein synthesis and detoxification, is one of the human body's most modeled organs. Because these cells are easy to handle, do not contain viruses, exhibit a high degree of morphological and functional differentiation *in vitro* (Almeida et al., 2022), and produce repeatable findings, the human hepatoma HepG2 cell line has been widely employed as an *in vitro* model of the human liver. HepG2 cells have been employed in pharmacological research

on drug targeting and carcinogenesis, as well as toxicological investigations on liver metabolism and the harmful effects of xenobiotics (Gupta et al., 2021). Gene expression profiling is an effective technique that has yielded a wealth of information in earlier research. Although transcriptome activity is often measured using microarrays, whole-transcriptome high-throughput sequencing offers the most comprehensive analysis, with a broad dynamic range and increased sensitivity (Han et al., 2015). A detailed experimental design is essential, given the high expense of conducting studies utilizing this technology.

Hyperglycemia is the end outcome of either inadequate insulin secretion, decreased insulin action, or a combination of the two in diabetes mellitus (DM), a chronic metabolic disease. Among the most common diseases of the twenty-first century is diabetes mellitus (DM), primarily brought on by a range of lifestyle issues (Kumar et al., 2020). Diabetes long-term hyperglycemia is a major contributor to blindness, kidney failure, heart attacks, strokes, and amputation of lower limbs. Millions of individuals worldwide are affected by diabetes mellitus (DM), which has turned into a global public health catastrophe (Wukich et al., 2022). The surge in type 2 DM incidence has raised concerns about global health. Insulin resistance, a condition where the pancreas generates insufficient or inefficient amounts of insulin, affects more than 90% of people with diabetes type 2. The metabolic condition known as type 2 diabetes mellitus (T2DM) is typified by hyperglycemia brought on by a comparatively low insulin level. Globally, the prevalence of type 2 diabetes is rising, raising serious health concerns (Shahwan et al., 2022). An International Diabetes Federation poll indicates that by 2040, more than 10% of adults worldwide are predicted to have type 2 diabetes (Teo et al., 2021). Insulin resistance and an insulin secretion abnormality are the primary causes of type 2 diabetes. In the early stages of type 2 diabetes, symptoms including mild fatigue and thirst are common in obese people with insulin resistance. Empirical evidence indicates that modifications to several genes and signaling pathways play a role in controlling the progression of type 2 diabetes (Zhu et al., 2023). Unfortunately, current research on the exact molecular processes of T2DM progression is lacking, which restricts the disease's therapy success. Therefore, for future non-invasive diagnosis and targeted therapy, it is critical to comprehend the molecular pathways of T2DM occurrence and development.

In the small intestine, glucose is created from carbohydrates and then enters the bloodstream. The enzymes α -amylase and α -glucosidase are crucial in facilitating the conversion of starch into glucose (Papoutsis et al., 2021). Only glucose transporters allow glucose to be absorbed

and used in the small intestine since it is unable to pass freely across the hydrophobic portion of the lipid layer of the cell membrane. Glucose transporters, including sodium-dependent glucose transporter 1, epithelium glucose transporter 2, and epithelial glucose transporter 5, are essential elements of the glucose transport process in numerous investigations (Gyimesi et al., 2020). As a result, the process of absorbing glucose in the small intestine depends critically on the expression levels of sodium-dependent glucose transporter 1, epithelium glucose transporter 2, and epithelial glucose transporter 5, and the proteins that are linked to them. Conventional therapy for type-2 diabetes entails blocking glucosidases like α -amylase and α -glucosidase from breaking down dietary starch. An oral antidiabetic medication called acarbose may prevent the actions of the enzymes α -amylase and α -glucosidase (Riyaphan et al., 2021). However, using these medications over an extended period of time might have a number of harmful effects, such as liver damage, indigestion, and bloating that were separated from *L. strychnifolium* for α -amylase and α -glucosidase in vitro investigations (Camilleri et al., 2017). Additionally, the impact of these substances on glucose transporters in human liver cell HepG2 and human intestinal epithelial Caco-2 cells was studied. The MTT assay was also used to assess the cytotoxicity and mRNA expression of genes related to glucose absorption. The comprehensive examination of differentially expressed genes (DEGs) in the development of type 2 diabetes is made possible by bioinformatic microarray analysis. Subsequently, we also analyzed the Gene Ontology (GO) functions (Shen et al., 2020).

4.2 Materials and methods:

4.2.1 Sample preparation:

Queen pineapples were collected from the local market of Tripura, India. Pineapple waste was cleaned, processed, and then dried at 35-40°C for 12–16 hours in an electric tray drier (Labotech, BDI–51, India). The dried powder was then obtained by grinding and sifting through a 100-mesh size sieve, and ethanol was used to de-oil the sample. All of the chemicals utilized, including the enzymes cellulase (3 U/mg) and xylanase (2500 U/g) were of analytical grade (SIGMA-ALDRICH Co.)

4.2.2 Extraction and Modification of queen pineapple waste

The dietary fiber was extracted from queen pineapple waste using ultrasound-assisted extraction by using extraction time 22.35 min, amplitude 46.9%, and solid: liquid ratio 1:27.5 under controlled conditions using a probe-type ultrasonicator (Model: Q700-200 Digital

Sonicator, Qsonica LLC, Make: India). The enzymatic modification of ultrasound-assisted extracted dietary fiber has been already done (Objective 3).

4.2.3 Anti-diabetic study of enzyme modified dietary fiber from queen pineapple waste

4.2.3.1 α -amylase inhibition activity:

With slight modifications, the α -amylase inhibitory activity of enzyme-modified dietary fiber (EMDF) derived from queen pineapple waste was assessed using the methodology described by Zheng et al., 2020. After preparing the EMDF to a concentration of 1–20 mg/mL, it was added to 500 μ L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl), along with 0.5 mg/mL of porcine pancreatic α -amylase (EC 3.2.1.1) and 290 U/mL of Sigma Aldrich. The mixture was then incubated for 10 minutes at 35°C. 500 μ L of 1% soluble starch solution prepared in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was then added to each tube as a substrate. After 10 minutes of incubation at 35°C, the reaction mixtures were stopped with 1.0 mL of the DNS solution. After being heated in a water bath for five minutes, the mixture was allowed to cool to room temperature. Concurrently, a control trial was established, devoid of the test specimen. At 540 nm, the absorbance was measured. The following formula was used to compute the % inhibition of the α -amylase inhibitory activity.

$$\text{Inhibition (\%)} = \frac{A(\text{control})_{540} - A(\text{EMDF})_{540}}{A(\text{control})_{540}} \times 100 \quad (1)$$

Where: A (control)₅₄₀ is the absorbance of the control at 540nm and A (EMDF)₅₄₀ is the absorbance of EMDF at different concentrations (1-20mg/ml) respectively.

4.2.3.2 α -glucosidase inhibition activity

The Zheng et al., 2020 method was slightly modified to investigate the inhibitory activity of EMDF on α -glucosidase. For ten minutes, a 100 μ l solution of porcine pancreatic α -glucosidase (EC 3.2.1.20) (15 U/mL) and EMDF ranging from 1 to 20 mg/mL concentration were incubated at 35°C. A 50 μ l solution of 0.1 M phosphate buffer (pH 6.9) containing 5 mM p-nitrophenyl- α -D-glucopyranoside was introduced. The mixes were incubated at 35°C for five minutes. Simultaneously, a control trial was established, without the test sample. At 405 nm, the absorbance was measured. The following formula was used to compute the % inhibition of α -glucosidase inhibitory activity.

$$\text{Inhibition (\%)} = \frac{A(\text{control})_{405} - A(\text{EMDF})_{405}}{A(\text{control})_{405}} \times 100 \quad (2)$$

Where: A (control)₄₀₅ is the absorbance of the control at 405 nm and A (EMDF)₄₀₅ is the absorbance of EMDF at 405nm with different concentrations (1-20mg/ml) respectively.

4.2.3.3 DPP IV inhibition

Using the DPP IV inhibition assay kit (MAK 203, Sigma-Aldrich, Germany) and the manufacturer's instructions, the DPP IV inhibition of enzyme-modified dietary fiber (EMDF) has been evaluated (Wang et al., 2015). The DPP IV inhibitor screening kit is effective in quickly identifying compounds that may block DPP IV, which releases fluorescent output 7-amino-4-methyl coumarin (AMC) when it cleaves the non-fluorescent substrate (H-Gly-Pro-AMC). Various EMDF doses ranging from 1 to 20 mg/ml were applied to each well, followed by the addition of 50 μ L of an inhibitory reagent mixture that contained 1 μ L of DPP IV enzymes and 49 μ L of DPP IV assay buffer solution. For every concentration of the chemicals, a blank control (that is, one without the enzyme) and an enzymatic control (one without the substance) were also made. After that, the wells were incubated at 37 °C for 10 minutes. Lastly, each reaction well containing the test sample, blank, and enzymatic control was filled with 25 mL of DPP IV assay buffer solution and DPP IV substrate. The ability of the compounds to cleave substrate and produce fluorescent product, evaluated at wavelengths of (λ_{ex} = 360 nm and λ_{em} = 460 nm), is related to the DPP IV enzyme inhibition potential. The investigated drugs' efficacy was contrasted with that of sitagliptin, a DPP IV inhibitor that is sold commercially.

4.2.4 Cell culture:

Caco2 and HepG2 cell line was obtained from the cell culture facility lab at QUB (Queen's University Belfast, UK). Each of the two cell lines was grown in MEM supplemented with 10% FBS, 1% penicillin-streptomycin solution, 1% L-glutamine, and humidity control (95% air, 5% CO₂) at 37 °C. After seven to eight days, the cells underwent differentiation, and during this time, they were given fresh medium every two to three days.

4.2.5 Cytotoxicity assessment by MTT assay in Caco2 and HepG2 cell line:

Caco2 and HepG2 cell lines were used in an MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazoliumbromide) based test to assess the in vitro cytotoxicity of enzyme-modified

dietary fiber (EMDF) from queen pineapple waste. In a 96-well plate, 2×10^6 cells were plated and let grow for 24 h. Different EMDF concentrations (0.1, 1, 2, 5, 10, 20 mg/mL) were applied to the cells for 24 h at 37°C. Then, each well was filled with 25 μ L of MTT (5 mg/mL in PBS), and it was incubated for three hours. After removing excess MTT and growth media, cells were rinsed with phosphate buffer saline (PBS) and treated with 200 μ L of dimethyl sulfoxide (DMSO) to break down the formazan crystals. Using a Multimode Microplate Reader, the absorbance at 570 nm was recorded to assess the purple hue formazan. The absorbance of untreated (medium alone) cells was used as the negative control, meaning that 100% of the cells were still viable, while the positive control (PC) consisted of cells that had been exposed to Sodium Azide, a recognized cytotoxic agent. Cell viability was measured and expressed as a percentage using the absorbance ratio of treated cells to that of untreated control cells.

4.2.6 Glucose (2-NBDG) uptake assay in Caco2 and HepG2 cell line:

The cell lines Caco2 and HepG2 were kept in the cell culture facility lab at Queen's University Belfast, UK. The cells were seeded into two distinct 96-well black plates, cultivated in 100 μ L of DMEM supplemented with 10% FBS, and incubated at 37 °C in a humid environment with 5% CO₂. 100 U of penicillin and 100 mg of streptomycin were added to one milliliter of DMEM. The cells known as myoblasts began to develop into myotubes two days after confluence. The DMEM medium containing 10% FBS, 10,000 units/mL penicillin, 25 g/mL amphotericin B, and 10 mg/mL streptomycin, together with 5% CO₂ at 37°C, was used to culture these myoblasts. After two to three passes of both cell lines' myoblasts, the cells were then allowed to develop in a humidified environment and fuse into myotubes in a culture medium containing 2% fetal bovine serum (FBS). Seven days following confluence, all experiments were performed on cells that had reached maximal differentiation (>85%). Under a microscope, the differentiation of myotubes was seen. Using a cellular model-based 2-NBDG glucose uptake test kit made by Cayman, USA, the glucose absorption in both cell lines was measured. The manufacturer's recommended technique was followed, as detailed by Luna-Vital and Gonzalez de Mejia (2018). The fluorescent-d-glucose analogue 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG) is directly cultivated in human cells for this 2-NBDG glucose uptake experiment. The fluorescence of the cells is then measured via flow cytometry. Myotubes were, in short, placed in DMEM without glucose for a whole day. Subsequently, the cells were cultured for an additional hour at different concentrations of EMDF (1–20 mg/ml), and then for a further four hours at 0.75 mM of palmitate. Thirty minutes

prior to the conclusion of the incubations, the cells were treated with insulin (100 nM). Before the experiment was concluded, the fluorescent label glucose analogue 2-NBDG was added to each of the incubations for a duration of 10 minutes. After cell lysis, a Multimode Microplate Reader (Varioskan LUX, Thermo Scientific, Finland) was used to detect the fluorescence intensity at $\lambda_{ex} = 485$ and $\lambda_{em} = 535$ nm. The result was displayed as mean fluorescence intensity (MFI).

4.2.7 RNA extraction of Caco2 and HepG2 cell line:

An RNA extraction kit (RNeasy Mini Kit, QIAGEN, USA) was used to extract RNA from Caco2 and HepG2 cells. SuperScript III Reverse Transcriptase (Thermo Fisher Scientific, Waltham, USA) was used for the reverse transcription process. Following the manufacturer's instructions, 2 μ g of total RNA was used to create cDNA. After that, the cDNA was kept at -20°C to continue.

4.2.8 RNAseq dataset and analysis:

Following RNA extraction, both cell lines underwent RNA-seq analysis. The entire sequencing procedure was completed at the UK Genomics Core Technology Unit (QUB Genomics CTU – PN0550) at Queen's University Belfast. NextSeq 2000 was the sequencing platform used for RNA sequencing, with a read length of two x 100 bp PE. FastQC was utilized for quality control, and readings with a quality score of $Q > 30$ were used for additional analysis. HTSeq was employed to analyze gene expression. Using transcript abundances as inputs to the DESeq (version 1.18.0) program, differential expression was evaluated. The transcripts classified as differentially expressed genes (DEGs) had $|\log_2 \text{fold change}| > \pm 1$ and $P < 0.05$. The genes that were significantly expressed in each sample were subjected to principal component analysis (PCA). Using topGO, the Gene Ontology (GO) enrichment analysis was carried out, and the primary biological activities of differential gene exercise were identified by calculating the P-value using the hypergeometric distribution approach to select the GO word that is considerably enriched. The associated signaling pathways were identified by counting the number of genes that were differentially expressed at each pathway.

4.2.9 Statistical analysis

For every experiment, there were three runs. Analysis of variance (ANOVA) was performed on the experimental results. Using Duncan's tests with SPSS 24 (IBM Analytics, USA),

substantial differences between the data were found, and a p-value < 0.05 was deemed statistically significant.

4.3 Results and discussion:

After enzymatic treatments of pineapple waste extracted dietary fiber achieved the desired modifications and the results showed the changes in the structural, thermal, and functional properties of enzyme-modified dietary fiber (EMDF) shown in objective 3. The EMDF was further investigated for its anti-diabetic properties.

4.3.1 Anti-diabetic study of enzyme-modified dietary fiber:

4.3.1.1 α -amylase inhibition:

The health-beneficial effects of enzyme-modified dietary fiber (EMDF) from queen pineapple waste were investigated for its antidiabetic potential using *in vitro* enzyme inhibitory assays. The *in vitro* α -amylase inhibition study revealed that EMDF showed significant α -amylase inhibition at all the tested concentrations (Fig. 4.1). Acarbose was used as a control and the inhibition activity exhibited 89.92% of α -amylase. 77.31% inhibition of α -amylase inhibitory activity was observed at 20mg/ml concentration of EMDF.

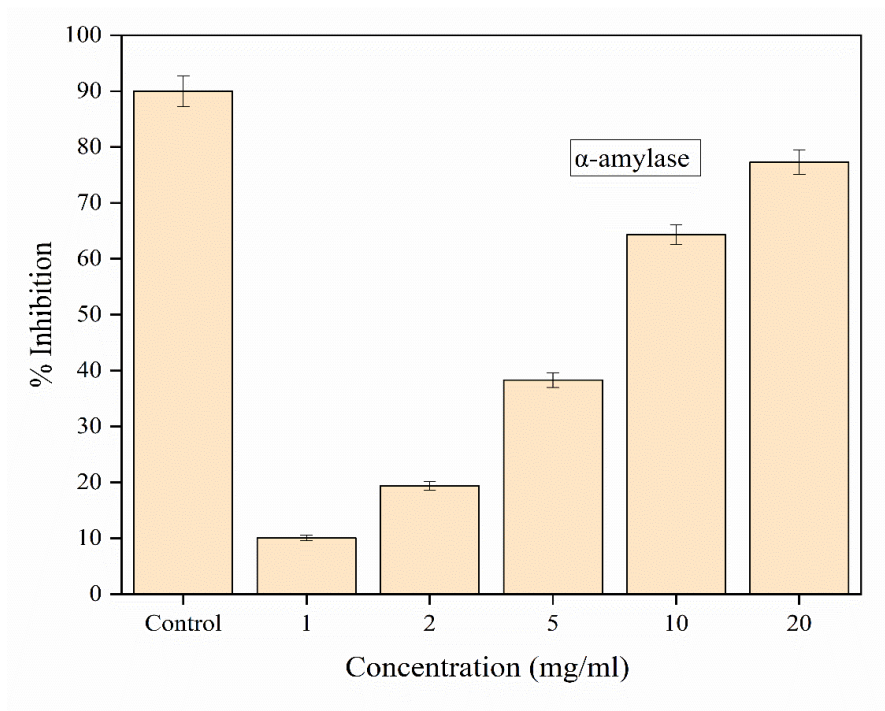


Fig. 4.1 : Inhibition of α -amylase activity

4.3.1.2 α -Glucosidase inhibition:

The *in vitro* α -glucosidase inhibition study revealed that EMDF showed significant α -glucosidase inhibition at all the tested concentrations. Acarbose exhibited a higher inhibitory effect on α -glucosidase (90.23%) as compared to the α -amylase enzyme. Generally, plant extract had higher inhibitory efficacy against α -glucosidase than α -amylase. Among all the concentration levels 20mg/ml concentration of EMDF exhibited the highest inhibition of over 81.23 % of α -Glucosidase enzyme inhibition (Fig. 4.2.). The results supported the beneficial effects of EMDF from queen pineapple waste for its antidiabetic potential.

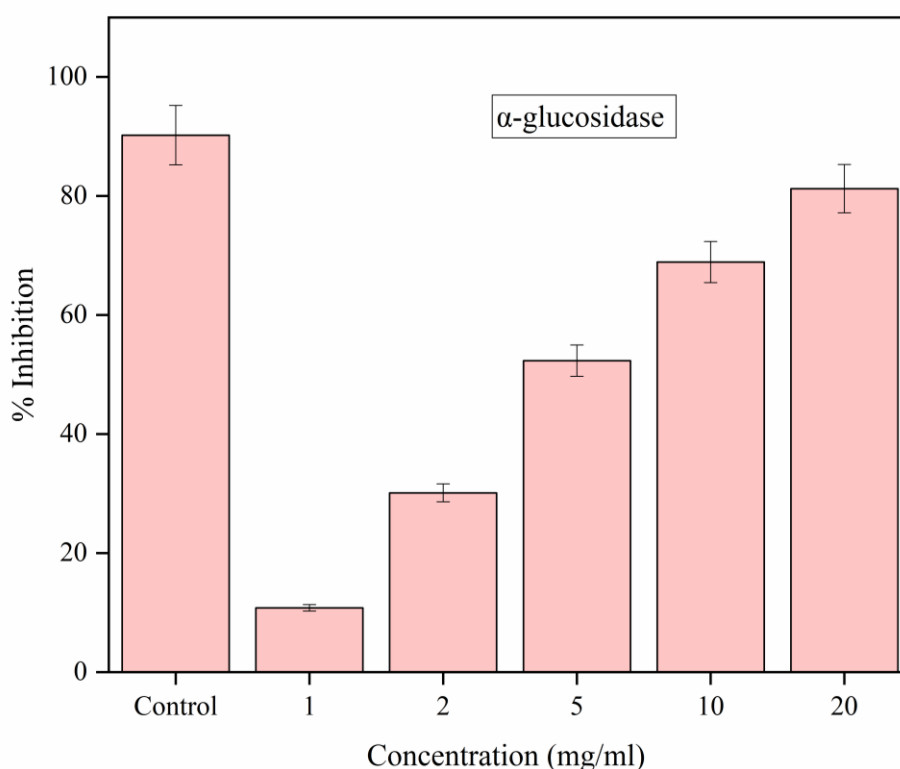


Fig. 4.2: Inhibition of α -amylase activity

Enzyme-modified dietary fiber (EMDF) has exhibited a substantial inhibitory effect on α -amylase and α -glucosidase activity, indicating its potential to regulate postprandial hyperglycemia. Interaction between dietary fibers and α -amylase or α -glucosidase reduces starch hydrolysis by converting rapidly digestible starch to slowly digestible starch and resistant starch fractions. The second mechanism is by directly inhibiting the activity of the enzymes. The interactions of insoluble and soluble dietary fibers (cellulose, hemicellulose, pectin, lignin, etc.) with one another and with other constituents played a role in the biological

effects that were observed since different fiber groups have different bioactivities (Tan et al., 2023). Dietary fiber is said to suppress α -amylase activity in two primary ways: (i) by reducing the accessibility between the enzyme and substrate by increasing the viscosity of the gastrointestinal mucus, or by encasing the enzyme and substrate in a fiber network structure; (ii) Dietary fiber can attach itself to the enzyme to create an inhibitor-enzyme or inhibitor-substrate complex, which will change the shape of α -amylase and prevent the enzyme from working (Zheng, et al., 2022).

4.3.1.3 DPP IV inhibition:

The DPP IV inhibitory potential of EMDF in the concentration varying from 1-20 mg/mL was examined in the present study, and was compared with sitagliptin used as a positive control, a commercially available DPP IV inhibitor drug, and the results are depicted in Fig. 4.3. EMDF containing a mixture of IDF and SDF produced a concentration-dependent inhibition of DPP IV.

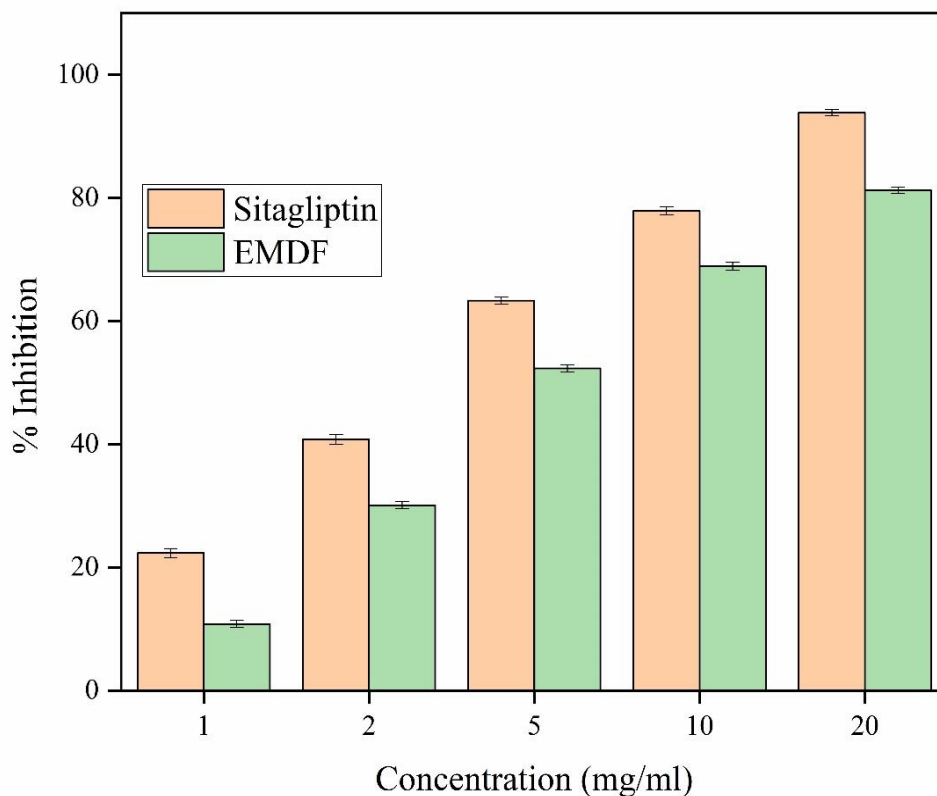


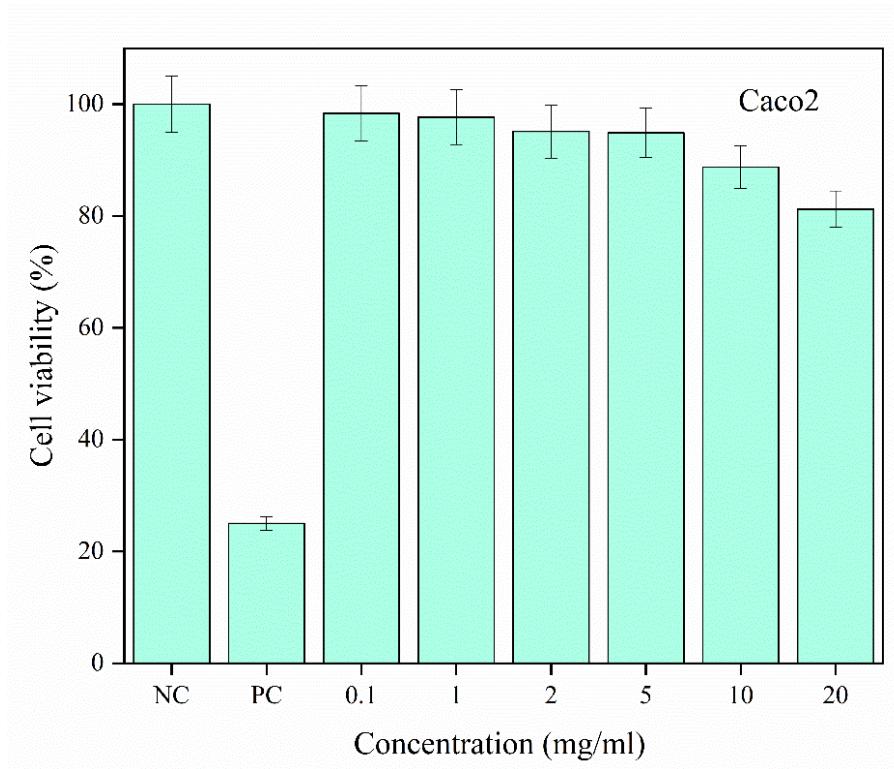
Fig. 4.3: Inhibition of DPP IV enzyme activity

The highest inhibition was attained at the highest concentration of both drugs. EMDF strongly inhibited the DPP IV enzyme (81.23%) at 20 mg/mL. Whereas, DPP IV inhibition potential of sitagliptin, a commercial anti-diabetic drug was 93.87% at 20 mg/ml. The extracts and the tested drug demonstrated significant differences in their inhibition potential. The presence of IDF and SDF in EMDF might be responsible for the observed effect. However, the remarkably high DPP IV inhibitory potential of Sitagliptin might be attributed to the purity and specificity of its tripeptide structure (Hannan et al., 2020). Thus, the outcomes of the DPP IV enzyme inhibition assay *in vitro* provided a rationale for the use of enzyme-modified dietary fiber from queen pineapple waste to manage diabetes mellitus. Naturally occurring DPP IV inhibitors, such as berberine were successful in inhibiting the enzyme DPP IV. The results supported the beneficial effects of EMDF which helps to indirectly lower postprandial blood sugar and finally its antidiabetic potential (Zabidi et al., 2021).

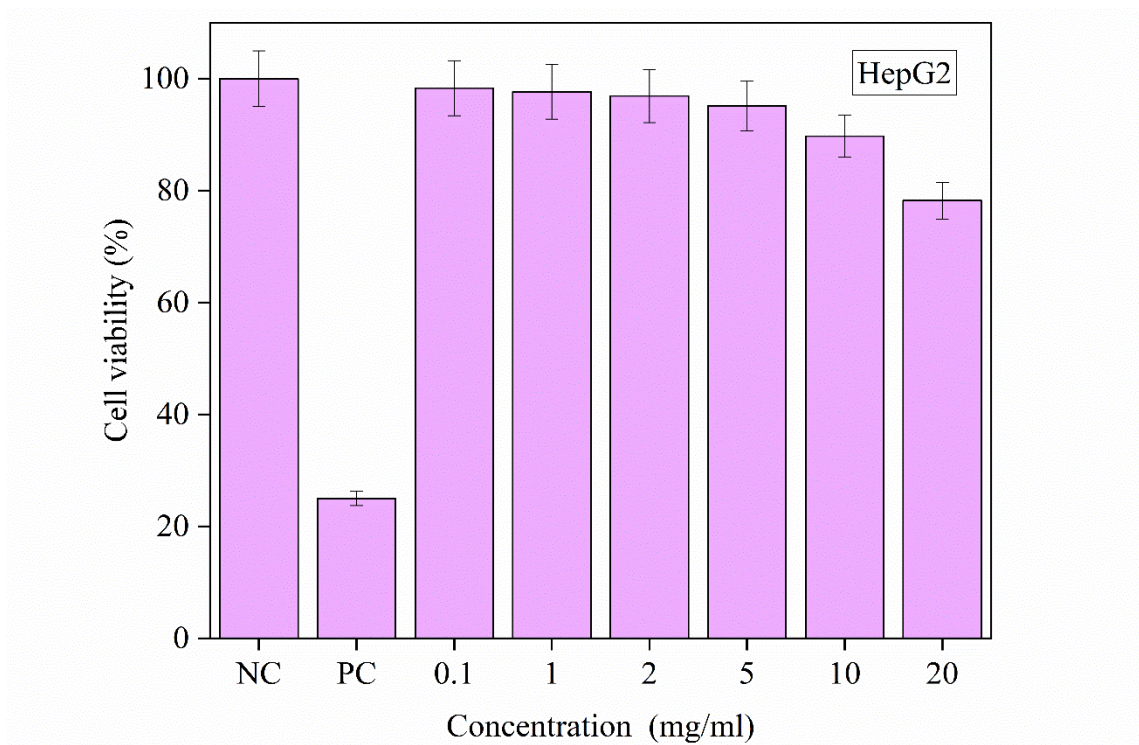
4.3.2 Toxicity study of enzyme-modified dietary fiber from queen pineapple waste

4.3.2.1 Cell viability assay of enzyme-modified dietary fiber in Caco2 and HepG2 cell line

The effects of different concentrations of EMDF on MTT-based cell viability assay in Caco 2 (human intestinal cells) and HepG2 (human liver cells) cell line was observed. These human cell lines were viable at a tested dose of 0.1, 1, 2, 5, 10, and 20 mg per ml of solution for EMDF incubated for 24 h (Fig. 4.4). The result shows that EMDF is not toxic to both the human intestinal and liver cells. The percentage of viable cells between control cells (untreated) and cells that were treated with EMDF (0.1 to 20 mg/mL) were examined for cytotoxicity. The cells treated with enzyme-modified dietary fiber did not show cytotoxic effects at any of the tested concentrations, which exhibited more than 80% cell viability. Positive control (PC): cells with known cytotoxic agent (Sodium Azide); Negative control (NC): cells without any treatment



(a)

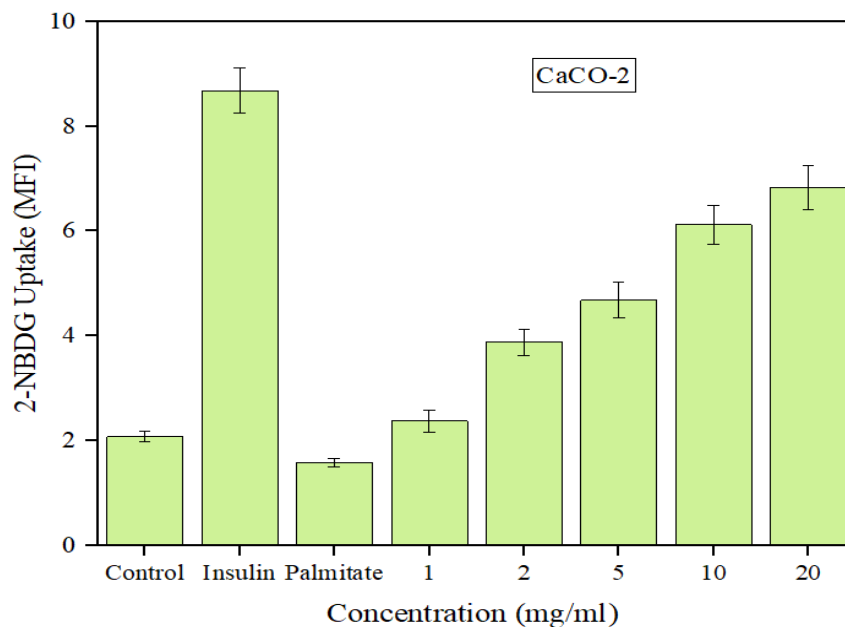


(b)

Fig. 4.4 Cell viability of (a) Caco2 (b) HepG2 cell line treated with of EMDF

4.3.3 *In vitro* glucose uptake assay of enzyme-modified dietary fiber

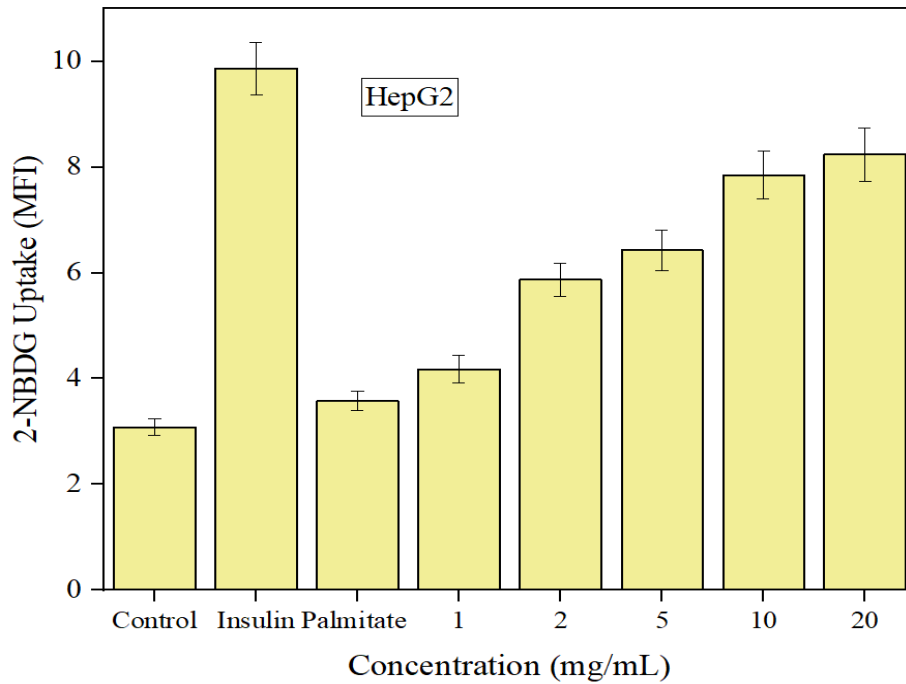
The effect of enzyme-modified dietary fiber (EMDF) treatment at the non-toxic concentrations of 1-20 mg/mL on 2-NBDG uptake in Caco2 and HepG2 cell lines were studied. DMSO was present in 0.1% concentration in each sample, including the control. The outcomes of the investigations are displayed in Fig. 4.5. It was observed that EMDF could successfully enhance glucose uptake when compared to the vehicle control. It was observed that 2-NBDG uptake escalated when the amount of EMDF increased. Our finding is consistent with the report of increased 2-NBDG uptake in Caco2 and HepG2 lines with higher concentrations (Lamm et al., 2015, Zakłós-Szyda et al., 2019).



(a)

Both the cell lines treated with insulin + palmitate were unable to absorb 2-NBDG, due to their severe Type 2 DM condition. The significant 2-NBDG uptake by EMDF-treated Caco2 and HepG2 cells suggests that it can repair injured cells. Our result is consistent with previous studies that exhibited anti-diabetic effects of EMDF. Therefore, EMDF may be utilized as a natural source of glucose uptake stimulant. It is noteworthy to mention that enzyme-modified dietary fiber from queen pineapple waste has not previously been reported to improve glucose absorption. Cellulose, hemicellulose, lignin, pectin, etc. which are abundantly present in EMDF, have already been demonstrated to facilitate glucose uptake by promoting migration of GLUT4 and enhanced function of AMP triggered protein kinase in Caco2 and HepG2 (McKim

et al., 2014). These effects may have resulted from their symbiotic interactions as it contain both IDF and SDF as bioactive compounds and exert their pharmacological behavior (Yu et al., 2023). These observed pharmacological behaviors might have been brought about by the synergistic interactions of several bioactive compounds (He et al., 2020).



(b)

Fig 4.5: Glucose uptake assay on (a) Caco2 and (b) HepG2 cells

2-NBDG = (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) Amino)-2-Deoxyglucose)

4.3.4 Effect of EMDF on RNA sequencing and differentially expressed mRNAs

An analysis of differentially expressed genes showed that there was a significant difference in the expression of 2389 genes between the treatment and control groups ($|\log_2 \text{fold change}| > \pm 1$, $P < 0.05$). The \log_2 fold change plot of DEGs shows that the genes that were up-regulated were 1298 and 1091, respectively. Fig. 4.6 shows the horizontal representation of gene in terms of heat map. Red genes indicate high expression and black genes indicate low expression; one sample per column.

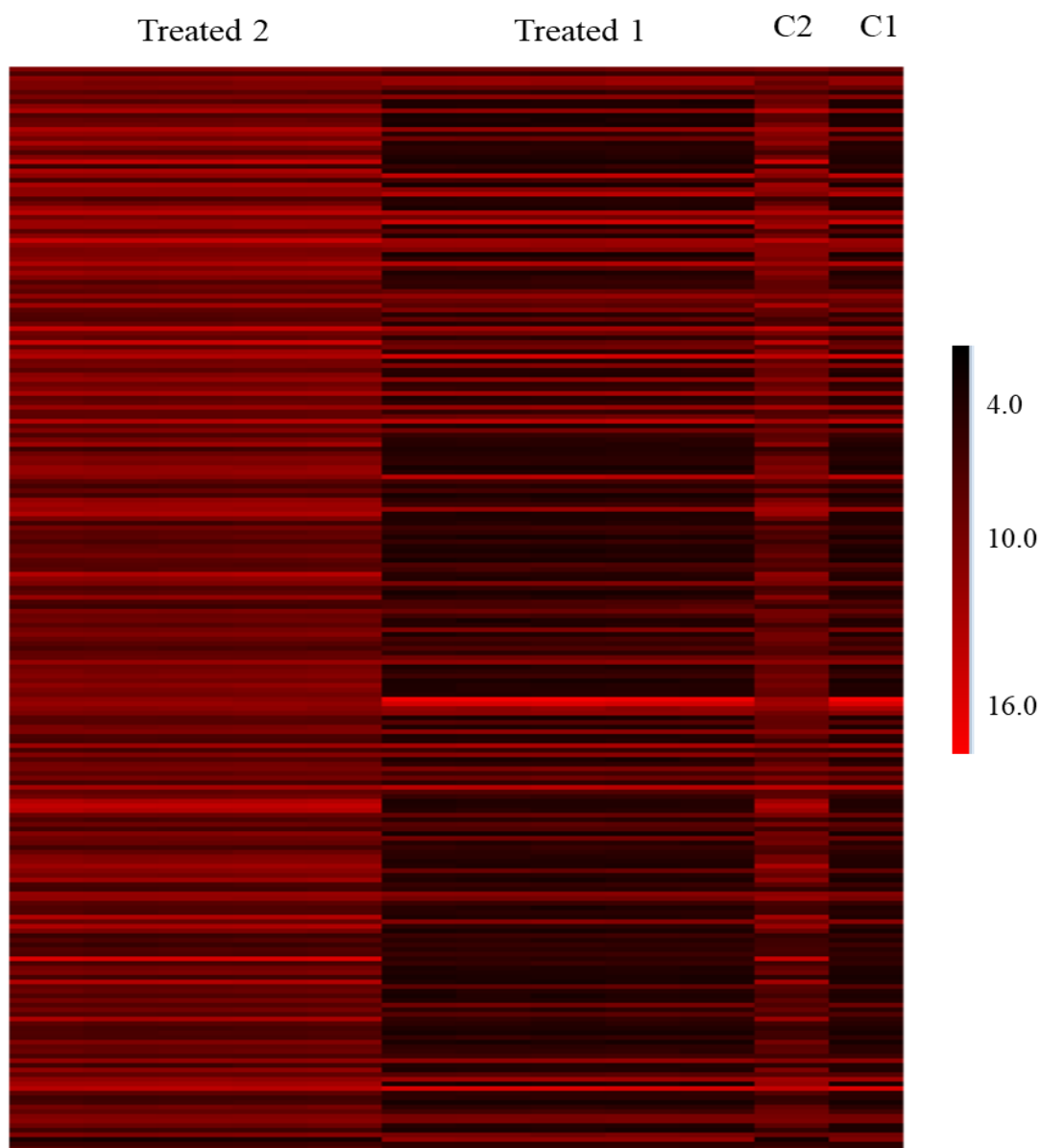


Fig. 4.6 The heatmap representation of differentially expressed genes changed mRNA transcripts

4.4.5 Functional prediction analysis of RNAseq by GO enrichment

The Gene Ontology (GO) enrichment analysis first mapped all the differentially expressed genes to each term in the GO database, Using the entire genome as the backdrop,. Then, it computed the significantly enriched terms of the DEGs using a hypergeometric distribution. Through GO functional enrichment analysis, a GO term with a significant enrichment of DEGs and linked biological functions can be derived. The results of DEGs' GO enrichment study were categorized based on biological processes, cellular functions, and molecular functions.

The GO terms in each GO classification with the greatest significant enrichment and the lowest p-value are displayed in Fig. 4.7.

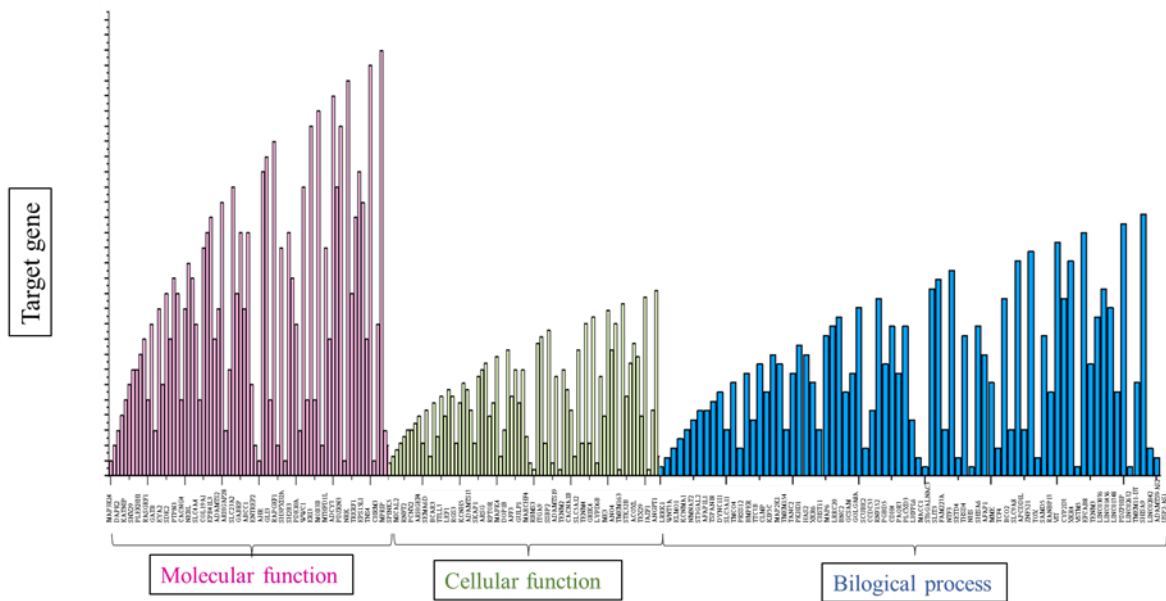


Fig. 4.7 GO function analysis of sDEGs into molecular function, cellular function, and biological process

The bar graph displays the three GO ontologies based on the findings of the enrichment analysis. The p-value and the ratio of DEGs to all genes in the term are indicated by the numbers under the GO term function, respectively. The most notable enrichment in the relatively generalized GO term of biological process (BP) is the immune system process, as illustrated in Fig. 4.8 of the biological process. Notable BP terms included defense response, positive regulation of the immune system, positive regulation ion transport, innate immune response, and response to external stimulus. The phrases "cell surface," "extracellular region part," "extracellular space," "multicellular organismal homeostat," "regulated exocytosis," and "endopeptide regulatory activity" were also significantly enriched in terms of cell function (CF), as shown in Fig. 4.8. In terms of molecular function (MF), protein binding, carbohydrate binding, and receptor binding were the top three terms.

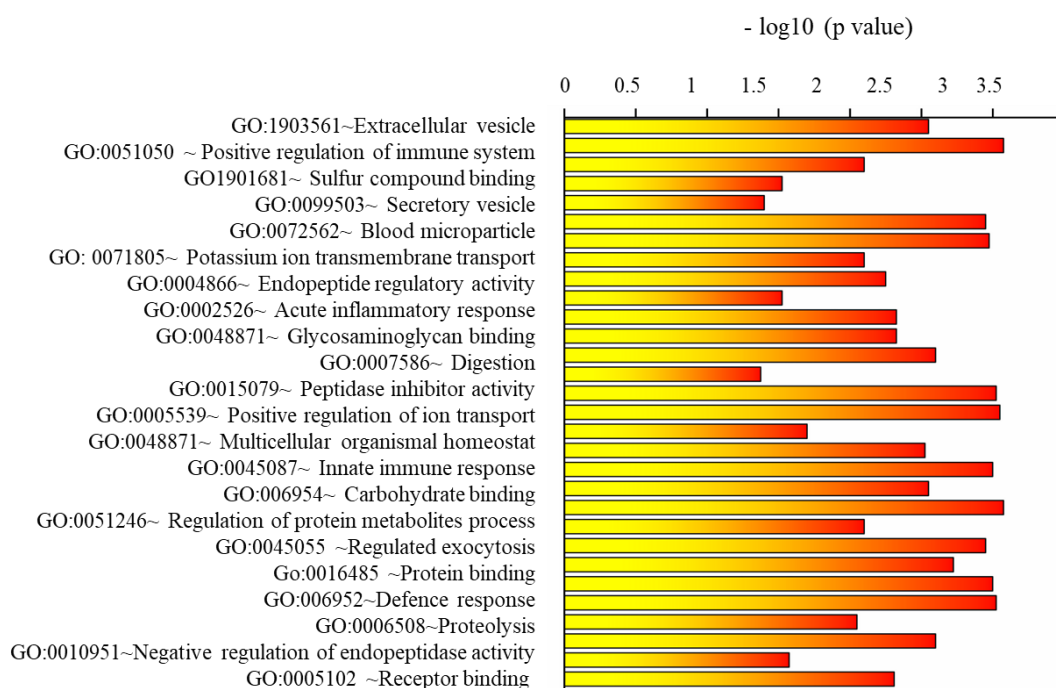


Fig. 4.8 The significant GO enrichment analysis of DEGs in different functional groups

4.4 Conclusion:

The study revealed that enzyme-modified dietary fiber (EMDF) from queen pineapple waste was effective for α -glycosidase, and α -amylase inhibition. The EMDF also showed strong inhibitory activity against DPP IV enzymes. After the *in vitro* toxicity study, it was also revealed that EMDF is nontoxic to human cell lines (Caco2 and Hepg2). The *in vitro* antidiabetic study revealed the health-beneficial potential of EMDF at different concentrations. An effective and popular molecular biology technique called RNA sequencing, or RNA-seq, offers previously unheard-of information about an organism's transcriptional landscape. 2389 differentially expressed genes (DEGs) were identified by RNA-seq analysis between the treatment and control groups. The up- and down-regulated genes were 1298 and 1091, respectively. To examine the impact of the treated material on immunological function after it enters the human intestine and liver cell lines, as well as any potential molecular mechanisms, GO enrichment analysis was carried out following the DEGs found.

Bibliography:

1. Almeida, J. I., Tenreiro, M. F., Martinez-Santamaria, L., Guerrero-Aspizua, S., Gisbert, J. P., Alves, P. M., ... and Baptista, P. M. (2022). Hallmarks of the human intestinal microbiome on liver maturation and function. *Journal of Hepatology*, 76(3), 694-725.
2. Camilleri, M., Lembo, A., and Katzka, D. A. (2017). Opioids in gastroenterology: treating adverse effects and creating therapeutic benefits. *Clinical Gastroenterology and Hepatology*, 15(9), 1338-1349.
3. Fedi, A., Vitale, C., Ponschin, G., Ayehunie, S., Fato, M., and Scaglione, S. (2021). In vitro models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review. *Journal of Controlled Release*, 335, 247-268.
4. Gupta, R., Schrooders, Y., Hauser, D., van Herwijnen, M., Albrecht, W., Ter Braak, B., ... and Caiment, F. (2021). Comparing in vitro human liver models to in vivo human liver using RNA-Seq. *Archives of Toxicology*, 95, 573-589.
5. Gyimesi, G., Pujol-Giménez, J., Kanai, Y., and Hediger, M. A. (2020). Sodium-coupled glucose transport, the SLC5 family, and therapeutically relevant inhibitors: from molecular discovery to clinical application. *Pflügers Archiv-European Journal of Physiology*, 472(9), 1177-1206.
6. Han, Y., Gao, S., Muegge, K., Zhang, W., and Zhou, B. (2015). Advanced applications of RNA sequencing and challenges. *Bioinformatics and Biology Insights*, 9, BBI-S28991.
7. Hannan, J. M. A., Ansari, P., Azam, S., Flatt, P. R., and Wahab, Y. H. A. (2020). Effects of *Spirulina platensis* on insulin secretion, dipeptidyl peptidase IV activity, and both carbohydrate digestion and absorption indicate potential as an adjunctive therapy for diabetes. *British Journal of Nutrition*, 124(10), 1021-1034.
8. He, J., Zhang, P., Shen, L., Niu, L., Tan, Y., Chen, L., ... and Zhu, L. (2020). Short-chain fatty acids and their association with signalling pathways in inflammation, glucose and lipid metabolism. *International Journal of Molecular Sciences*, 21(17), 6356.
9. Kumar, R., Saha, P., Kumar, Y., Sahana, S., Dubey, A., and Prakash, O. (2020). A review on diabetes mellitus: type1 & Type2. *World Journal of Pharmacy and Pharmaceutical Sciences*, 9(10), 838-850.
10. Lammi, C., Zanoni, C., and Arnoldi, A. (2015). Three peptides from soy glycinin modulate glucose metabolism in human hepatic HepG2 cells. *International Journal of Molecular Sciences*, 16(11), 27362-27370.

11. Luna-Vital, D. A., and Gonzalez de Mejia, E. (2018). Anthocyanins from purple corn activate free fatty acid-receptor 1 and glucokinase enhancing in vitro insulin secretion and hepatic glucose uptake. *PloS one*, 13(7), e0200449.
12. McKim, J. M. (2014). Food additive carrageenan: Part I: A critical review of carrageenan in vitro studies, potential pitfalls, and implications for human health and safety. *Critical Reviews in Toxicology*, 44(3), 211-243.
13. Motta, J. P., Wallace, J. L., Buret, A. G., Deraison, C., and Vergnolle, N. (2021). Gastrointestinal biofilms in health and disease. *Nature Reviews Gastroenterology & Hepatology*, 18(5), 314-334.
14. Pamies, D., Estevan, C., Vilanova, E., and Sogorb, M. A. (2022). Alternative methods to animal experimentation for testing developmental toxicity. In *Reproductive and Developmental Toxicology* (pp. 107-125). Academic Press.
15. Papoutsis, K., Zhang, J., Bowyer, M. C., Brunton, N., Gibney, E. R., and Lyng, J. (2021). Fruit, vegetables, and mushrooms for the preparation of extracts with α -amylase and α -glucosidase inhibition properties: A review. *Food Chemistry*, 338, 128119.
16. Ranganathan, S., Smith, E. M., Foulke-Abel, J. D., and Barry, E. M. (2020). Research in a time of enteroids and organoids: how the human gut model has transformed the study of enteric bacterial pathogens. *Gut Microbes*, 12(1), 1795389.
17. Riyaphan, J., Pham, D. C., Leong, M. K., and Weng, C. F. (2021). In silico approaches to identify polyphenol compounds as α -glucosidase and α -amylase inhibitors against type-II diabetes. *Biomolecules*, 11(12), 1877.
18. Schoultz, I., and Keita, Å. V. (2020). The intestinal barrier and current techniques for the assessment of gut permeability. *Cells*, 9(8), 1909.
19. Sensoy, I. (2021). A review on the food digestion in the digestive tract and the used in vitro models. *Current Research in Food Science*, 4, 308-319.
20. Shahwan, M., Alhumaydhi, F., Ashraf, G. M., Hasan, P. M., and Shamsi, A. (2022). Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *International Journal of Biological Macromolecules*, 206, 567-579.
21. Shen, Z., Chen, Q., Ying, H., Ma, Z., Bi, X., Li, X., ... and Fu, G. (2020). Identification of differentially expressed genes in the endothelial precursor cells of patients with type 2 diabetes mellitus by bioinformatics analysis. *Experimental and Therapeutic Medicine*, 19(1), 499-510.
22. Tan, Y., Li, S., Li, C., and Liu, S. (2023). Glucose adsorption and α -amylase activity inhibition mechanism of insoluble dietary fiber: Comparison of structural and

- microrheological properties of three different modified coconut residue fibers. *Food Chemistry*, 418, 135970.
23. Teo, Z. L., Tham, Y. C., Yu, M., Chee, M. L., Rim, T. H., Cheung, N., ... and Cheng, C. Y. (2021). Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. *Ophthalmology*, 128(11), 1580-1591.
 24. Wang, F., Yu, G., Zhang, Y., Zhang, B., and Fan, J. (2015). Dipeptidyl peptidase IV inhibitory peptides derived from oat (*Avena sativa* L.), buckwheat (*Fagopyrum esculentum*), and highland barley (*Hordeum vulgare trifurcatum* (L.) Trofim) proteins. *Journal of Agricultural and Food Chemistry*, 63(43), 9543-9549.
 25. Wukich, D. K., Raspovic, K. M., Jupiter, D. C., Heineman, N., Ahn, J., Johnson, M. J., ... and Nakonezny, P. A. (2022). Amputation and infection are the greatest fears in patients with diabetes foot complications. *Journal of Diabetes and its Complications*, 36(7), 108222.
 26. Yu, C., Dong, Q., Chen, M., Zhao, R., Zha, L., Zhao, Y., ... and Ma, A. (2023). The effect of mushroom dietary fiber on the gut microbiota and related health benefits: a review. *Journal of Fungi*, 9(10), 1028.
 27. Zabidi, N. A., Ishak, N. A., Hamid, M., Ashari, S. E., and Mohammad Latif, M. A. (2021). Inhibitory evaluation of *Curculigo latifolia* on α -glucosidase, DPP (IV) and in vitro studies in antidiabetic with molecular docking relevance to type 2 diabetes mellitus. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 36(1), 109-121.
 28. Zakłós-Szyda, M., Pawlik, N., Polka, D., Nowak, A., Koziółkiewicz, M., and Podsędek, A. (2019). *Viburnum opulus* fruit phenolic compounds as cytoprotective agents able to decrease free fatty acids and glucose uptake by Caco-2 cells. *Antioxidants*, 8(8), 262.
 29. Zheng, Y., Xu, B., Shi, P., Tian, H., Li, Y., Wang, X., ... and Liang, P. (2022). The influences of acetylation, hydroxypropylation, enzymatic hydrolysis and crosslinking on improved adsorption capacities and in vitro hypoglycemic properties of millet bran dietary fibre. *Food Chemistry*, 368, 130883.
 30. Zhu, H., and Leung, S. W. (2023). MicroRNA biomarkers of type 2 diabetes: evidence synthesis from meta-analyses and pathway modeling. *Diabetologia*, 66(2), 288-299.