Chapter 3

To evaluate the effect of enzymatic modification of extracted dietary fiber and its characterization

3.1 Introduction

The Indian economy heavily relies on agriculture, and extracting bioactive compounds from agricultural residue could significantly boost national finances. Researchers have devised numerous innovative methods to minimize waste from crops, emphasizing the importance of utilizing agricultural residue and eco-friendly practices to preserve the environment. These bioactive compounds have diverse applications in pharmaceuticals, food and beverages, and biodegradable packaging, driving ongoing research efforts to harness them for ecological balance. Overall, leveraging bioactive compounds from agricultural waste presents a wide range of opportunities across industries, promising sustainable resource utilization and product innovation.

Pineapple (*Ananas comosus*), a non-climacteric fruit from the Bromeliaceae family, is renowned for its rich nutritional content and medicinal attributes (Dhar and Deka, 2023). Ranking third among tropical fruits worldwide, after bananas and citrus, pineapple offers versatile processing options, including canning and consumption fresh. Pineapple waste is notably abundant in bioactive compounds like dietary fiber (DF), polysaccharides, minerals, vitamins, enzymes, proteins, and volatile compounds, presenting potential applications across diverse industries (Dhar et al., 2023). Generated post-peeling and juice extraction, pineapple waste primarily consists of dietary fiber. Recent years have seen a surge in research on dietary fiber extraction and modification from fruit and vegetable waste.

The amount of non-starch polysaccharides that can either fully or partially ferment in the large intestine but resist enzymatic digestion in the small intestine is known as dietary fibre (DF). DF ranks as our body's 7th most vital nutrient (Hu et al., 2022). DF is categories into two types based on its solubility: soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). Pectin, gums, and other viscous plant polysaccharides are included in SDF, which is mostly utilised in products to enhance mouthfeel. Cellulose, hemicellulose, and lignin—the three main components of IDF—have potent qualities for binding, hydration, swelling, and oil retention. In addition to decreasing gastrointestinal transit time and increasing faecal size, IDF also lowers the risk of conditions like obesity, type-II diabetes mellitus, colon cancer, and cardiovascular disease. IDF's strong cation exchange capacity facilitates its detoxifying function, which is further enhanced by its free carboxyl and hydroxyl groups.

Through the use of modification procedures, insoluble dietary fibre (IDF) is converted into soluble dietary fibre (SDF), improving its physicochemical and physiological characteristics. These techniques include enzymatic treatments, chemical treatments, mechanical degradation,

and fermentation by microbes (Gan et al., 2021). In general, combining several techniques produces better results than using just one. For example, alterations including chemicals, ultrasounds, and microwaves have all been investigated (Garcia-Vaquero et al., 2020). By using enzymes to break down dietary fibre, enzymatic modification lowers the amount of IDF while increasing the amount of SDF. Enzymatic reactions operate under mild conditions with high specificity, resulting in minimal disruption to fiber composition and structure, and negligible chemical pollution, rendering them highly suitable for the food industry (Maghraby et al., 2023). Xylanase, cellulase, and lignin oxidase are among the primary enzymes employed in dietary fiber modification, with ongoing efforts aimed at refining enzymatic techniques.

Enzymes play a crucial role in extracting dietary fiber due to their remarkable efficiency, specificity, and gentle processing conditions. The major compounds of IDF are cellulose and hemicellulose, forming a dense polymeric structure (Sang et al., 2022). Consequently, various techniques are utilized to modify IDF, converting the polymeric matrix into simpler carbohydrates. These alterations can loosen the compact structure, improve the porosity, and ease partial degradation, thereby improving the physico-chemical and biochemical properties of IDF. Given the coarse texture and limited utilization potential of IDF, several methods have been employed for its modification. Carrot pomace IDF, treated with complex enzymes, demonstrated enhanced cholesterol adsorption capacities, antioxidant activities, and physico-chemical properties. Enzymatic treatment emerges as a straightforward, cost-effective, and environmentally friendly method for modification (Ayala-Zavala et al., 2018). Reports indicate that enzymatic hydrolysis significantly improves oil holding capacity (OHC), glucose adsorption capacities (GAC). Moreover, it has been noted that the water holding capacity (WHC), oil binding capacity (OBC), and expansibility of enzymatically treated modified dietary fiber are significantly enhanced compared to non-modified dietary fiber ($P < 0.05$) (Huang et al., 2021).

Insoluble dietary fibre (IDF) modification techniques and the physicochemical and biochemical characteristics of modified IDF (MIDF) have been the subject of recent research; yet, little is known about MIDF's possible applications (Jiang et al., 2023). The water retention capacity (WRC) and water solubility capacity (WSC) of modified dietary fibre are significantly higher than those of unmodified fibre when cellulase, a frequent enzyme in enzymatic modification, is added ($P < 0.05$). The breakdown of granular formations, which exposes more hydroxyl groups, is thought to be the cause of this improvement (Xie et al., 2019).

Dietary fiber also plays a crucial role in human health, and its modification through enzymatic treatments can enhance its functional properties. The present investigation aimed to examine the impact of cellulase/xylanase modification on the morphology and techno-functional aspects of DF derived from pineapple waste. Understanding these changes can help with the use of pineapple waste as a high-fiber component in culinary goods.

3.2 Methodology

3.2.1 Materials:

Queen pineapples were collected from a farm in Tripura, India. Pineapple waste was cleaned, processed, and then dried at 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 high-purity analytical grade (Sigma-Aldrich Co.).

3.2.2 Ultrasonic-assisted extraction of IDF:

The 5g dried sample was mixed with 60 mL of sodium hydroxide solution (NaOH) and phosphate buffer (PB) (PB: NaOH = 5:1 v/v, pH 7.5) (Łukajtis et al., 2018). After that, the mixture was subjected to ultrasonic extraction using a probe ultrasonicate (Q700-220 Digital Sonicator, Qsonica LLC, USA) for 30 minutes at 47% amplitude (Dhar and Deka; 2022). Eventually, the suspension was centrifuged for 20 minutes at 7000 rpm. Finally, the precipitate was taken out and given a distilled water wash. As IDF, the resultant precipitate was dried over night at 40 °C.

3.2.3 Modification of IDF:

The ultrasonic-assisted extracted dietary fiber (UAEDF) obtained was modified by using the methodology of Devi et al., 2023 with slight modification. Pineapple waste extracted dietary fiber was thoroughly blended with phosphate buffer solution (PBS) with a ratio of 1:20 (w/v) to achieve a pH of 4.9. Subsequently, a 0.18% solution (w/v, %) of cellulase:xylanase=1:1 was thoroughly mixed with the phosphate buffer and maintained at 50°C for hydrolysis in an oscillating water bath for 2 hours. To deactivate the enzymes, the mixture was boiled for 15 minutes. Precipitation was induced by adding fourfold volumes of 90% (w/w) ethanol, followed by 12 hours of filtration. After filtration, the residue was dried in a vacuum oven at

60ºC for 7 hours. The final product was enzyme-modified dietray fiber (EMDF) of pineapple waste.

3.2.4 FTIR analysis of modified dietary fibre

Fourier Transform Infrared spectroscopy was utilized to identify the various functional groups present in the enzyme-modified dietary fibre (EMDF). With a resolution of 4 cm^{-1}, the spectrum was studied throughout the 400–4000 cm^{-1} wavelength range (Dhar and Deka; 2023).

3.2.5 SEM analysis of modified dietary fibre

The samples (PDP, AEDF, UAEDF, EMDF) were studied by using Scanning Electron Microscope (JSM-6390LV, JEOL, Japan), operated at 20kV, 500X, and 1000X magnifications to analyze the microstructure and surface morphology.

3.2.6 XRD analysis of modified dietary fibre

The X-ray diffraction (Bruker AXS, Bruker D8 FOCUS, Germany) of EMDF was determined and XRD functioned at 60 kV. The study was executed at a 2θ range of 5-80° and the degree of crystallinity was calculated as follows:

$$C\ (\%)\ =\ \frac{X_c \times 100}{X_c + X_a} \tag{1}$$

Where, C is the degree of crystallinity; X_c is the crystalline area and X_a is the amorphous area on the XRD graph (Ma & Mu, 2016).

3.2.7 Thermo-gravimetry analysis (TGA) of modified dietary fibre

The thermal analysis of the samples (UAEDF, EMDF) was studied by using TGA (NETZCH TG 209F1 Libra, Germany) (Ren et al., 2021). The TGA was carried out under the controlled environment of nitrogen and a temperature range of 30°- 700°C at a rate of 10°C/min.

3.2.8 Functional characterization of the enzyme-modified dietary fibre

3.2.8.1 Water holding capacity (WHC) and oil holding capacity (OHC)

The water-holding capacity (WHC) and oil-holding capacity (OHC) were assessed following a methodology adapted from Ma & Mu (2015). Two grams of dietary fiber were mixed with either 20 ml of deionized water or vegetable oil, depending on whether WHC or OHC was

being measured, and agitated at 25°C for 30 to 35 minutes. The mixture underwent centrifugation at 5000 rpm for 15 minutes. WHC and OHC were then quantified as the weight gained by the dietary fiber per gram of dry weight, calculated using the provided equation.:

$$WHC \ or \ OHC \ (g/g) \ = \ (Wt - Wi) \ / \ Wi \qquad (2)$$

Where, W_t represents the final weight of DF and W_i represents the initial weight of DF.

3.2.8.2 Swelling capacity (SC)

A small modification was made to the method for analysing swelling capacity (SC) by Tan et al., (2017). A graduated glass cylinder was used to record the initial volume when the sample (1 g) was added. After adding 30 millilitres of phosphate buffer (pH 7.4), the samples were left to swell for 60 minutes. By taking the final volume of the swollen sample and expressing it as mL of final volume per g of starting dry sample, the swelling capacity of the sample was calculated.

3.2.8.3 Emulsifying activity (EA) and Emulsion stability (ES)

After adding 5g of the sample to a 1:2 ratio of deionized water, 5 mL of refined oil was sonicated for 10 minutes. Next, the mixture was centrifuged for five minutes at 4000 rpm. In the end, the tube's entire contents as well as the height of the emulsified layer were determined (Dhar and Deka; 2023). The EA was calculated as follows:

$$EA(\%) \ = \ (Height \ of \ the \ emulsified \ layer \ )/(Height \ of \ total \ content \ ) \times 100 \qquad (3)$$

The emulsion was heated to 80°C for 40 minutes, and then centrifugation at 5000 rpm for 10 minutes was used to assess the stability of the emulsion. The ES was determined as follows:

$$ES \ (\%) \ = \ (Height \ of \ the \ emulsified \ layer \ after \ heating)/$$
$$(Height \ of \ emulsified \ layer \ before \ heating \ ) \times 100 \qquad (4)$$

3.2.8.4 Cation exchange capacity (CEC)

The dietary fibre (UAEDF and EMDF) sample was treated with 2M HCl for 48 hours in order to determine the cation exchange capacity (CEC). After that, 0.5g of the wet sample was extracted, and 100 ml of 5% NaCl in buffer (pH 7.4) was added. The mixture was then filtered.

Following a 60-minute shaking and incubation period at 37°C, the sample was titrated, and the final results were reported as meq per g of the initial dry sample (Dhar and Deka; 2022).

3.2.8.5 Cholesterol absorption capacity (CAC)

The method used to determine cholesterol absorption capacity (CAC) was outlined in a prior work by Wu et al., 2020 with slight modification. A 10 mL test tube was filled successively with volumes of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mL of a cholesterol standard solution with a concentration of 0.01 mg/mL. Following addition of acetic acid and reaction with 2 mL of ammonium ferric sulfate reagent, total volume was adjusted to 4 mL. For the standard curve, the wavelength of the resulting solution was measured at 570 nm. Fresh egg yolks were separated and emulsified in 9 volumes of distilled water. Following homogenization, the emulsion's pH was initially set to 2.0 and then adjusted to neutral. Subsequently, samples were introduced into the emulsion and allowed to absorb cholesterol at 37°C for two hours. After centrifugation for five minutes at 5000 rpm, the absorbance of the supernatant was determined at 570 nm.

3.2.9 Hypoglycemic activity

3.2.9.1 Glucose adsorption capacity (GAC)

With a slight modification, the glucose adsorption capacity (GAC) was measured using the method by Dhar and Deka (2023) method. Individual 1g sample (UAEDF, and EMDF) was mixed with 25 millilitres of a glucose solution with concentrations of 10, 50, 100, and 200 mM. A magnetic stirrer was used to stir the mixture, and it was then incubated for six hours at 400 rpm in a water bath at 37°C. After centrifugation for 20 minutes at 5000 rpm, the amount of glucose in the supernatant was measured. The following formula was used to estimate the glucose adsorption capacity:

$$GAC \ (mmol/g) \ = \ ((G1 - G6))/ \ ((Ws \times Vi)) \tag{5}$$

Where, G1 represents initial glucose concentration in mmol/L; G6 represents final glucose concentration after 6 h in mmol/L; Vi is the volume of supernatant in ml; WS is the weight of dietary fibre in g.

3.2.9.2 Glucose dialysis retardation index (GDRI)

The GDRI analysis is a technique employed to assess glucose absorption within the 30–180 minute timeframe in the human gastric intestinal tract. To conduct the GDRI determination, the method outlined by Dhar and Deka (2023) was adopted. Each sample consisted of 0.5g of dietary fiber (DF) enclosed in a dialysis membrane bag with a molecular weight cutoff of 12,000 Da, along with 25mL of a glucose solution at a concentration of 50 mmol/L. Dialysis was performed individually for each bag using 100 mL of distilled water (DW) at 37°C, with gentle agitation at 50 rpm. To track glucose retardation over time, the glucose assay kit (Sigma GAGO-20) was employed to quantify the glucose concentration in the dialysate. Absorbance measurements were taken at 30, 60, 120, and 180 minutes after the diffusion of 1 mL of glucose from both the treated samples (UAEDF, EMDF) and the control (samples without the addition of DF).The GDRI was determined using the following equation:

$$GDRI = 100 - [(Gts/Gc) \times 100] \tag{6}$$

Where, G_{ts} represents the total glucose diffused from the treated sample; G_c represents the total glucose diffused from the control sample.

3.2.10 Statistical analysis

Statistical analysis of the data was performed using SPSS version 16.0. Mean values were assessed using analysis of variance (ANOVA), followed by Duncan's multiple range tests to determine significant differences ($p < 0.05$).

3.3 Result and discussion:

Enzymatic treatments with cellulase and xylanase were used to achieve the desired modifications in the dietary fiber. The enzymatic modification of pineapple waste dietary fiber resulted in changes in its total yield, structural, thermal, and functional properties. The chemical composition was also altered, leading to enhanced solubility and porosity of the dietary fiber. These changes were attributed to the disruption of intermolecular bonds and alterations in the crystalline structure.

The moisture-corrected values of total, insoluble, and soluble dietary fiber extracted from pineapple waste are presented in Table 3.1. The use of ultrasonic-assisted enzymatic hydrolysis notably enhanced the extraction efficiency of total dietary fiber (TDF), increasing it from

64.43±0.41% to 87.67±0.25%. The application of ultrasonic power amplifies both mechanical action and cavitation effects, facilitating thorough enzyme-sample interactions. This promotes cell rupture, accelerating starch hydrolysis, and prompts protein hydrolysis with the addition of NaOH solution. Complete separation of hemicellulose and cellulose increases TDF extraction. The enzyme-modified dietary fiber exhibited a significantly higher soluble dietary fiber (SDF) content (5.33±0.19%) compared to unmodified fiber (4.33±0.18%). This could be attributed to the conversion of insoluble cellulose and hemicellulose into soluble cell wall polysaccharides by cellulase and xylanase enzymes. Previous research by Wang et al. (2020) indicated that the conversion of insoluble dietary fiber (IDF) to SDF during additional SDF formation was facilitated by trans-glycosylation. These findings strongly support the notion that cellulase/xylanase treatment effectively promotes the conversion of IDF to SDF.

Table 3.1: The dietary fiber groups in pineapple waste

Dietary fiber	Conventional extraction (CE)	Ultrasonic-assisted extraction (UAE)	Enzyme modification (EM)
Total dietary fiber (TDF) (%)	64.43±0.41[c]	80.11±0.70[c]	87.67±0.25[abc]
Insoluble dietary fiber (IDF) (%)	62.89±0.21[a]	75.43±0.46[b]	82.34±0.28[ab]
Soluble dietary fiber (SDF) (%)	1.54±0.38[b]	4.68±0.18[a]	5.33±0.19[a]

3.3.1 FTIR of modified dietary fibre:

Functional group compositions are frequently investigated using FTIR spectroscopy, which is well-known for its sensitivity to changes in molecular structures (Gawkowska et al., 2018). The dietary fiber showed distinctive polysaccharide absorption peaks, which appeared as broad, intense bands between 3200 and 3600 cm^{-1}, as shown in Fig. 3.1. These peaks showed the bonding between hydrogen and hydroxyl groups in cellulose and hemicellulose, and they correlated with the tensile vibration of O-H in alcohol, phenol, and other hydrogen bonds (Jiang et al., 2018). Furthermore, the wide band demonstrated the existence of hemicellulose, which included galactose, xylose, and arabinose, as well as pectin. The polysaccharide methylene group's C–H vibration bands stretched, resulting in the absorption peak at 2820 cm^{-1}.

Enzymatic treatment decreased the band's intensity, indicating that the polysaccharides' hydroxyl groups' hydrogen bonds were disrupted. Absorption bands of 1000–1900 cm⁻¹, according to Ognyanov et al. (2020), suggested the stretching of methoxy glucuronic acid in hemicellulose and carboxyl groups in pectin's galactose. Notably, at 1600 cm⁻¹ and 1670 cm⁻¹, UAEDF and EMDF displayed the typical absorption C–O stretching vibrations. These vibrations are attributed to the esterified carboxyl group in lignin and, in the case of the former, aromatic benzene.
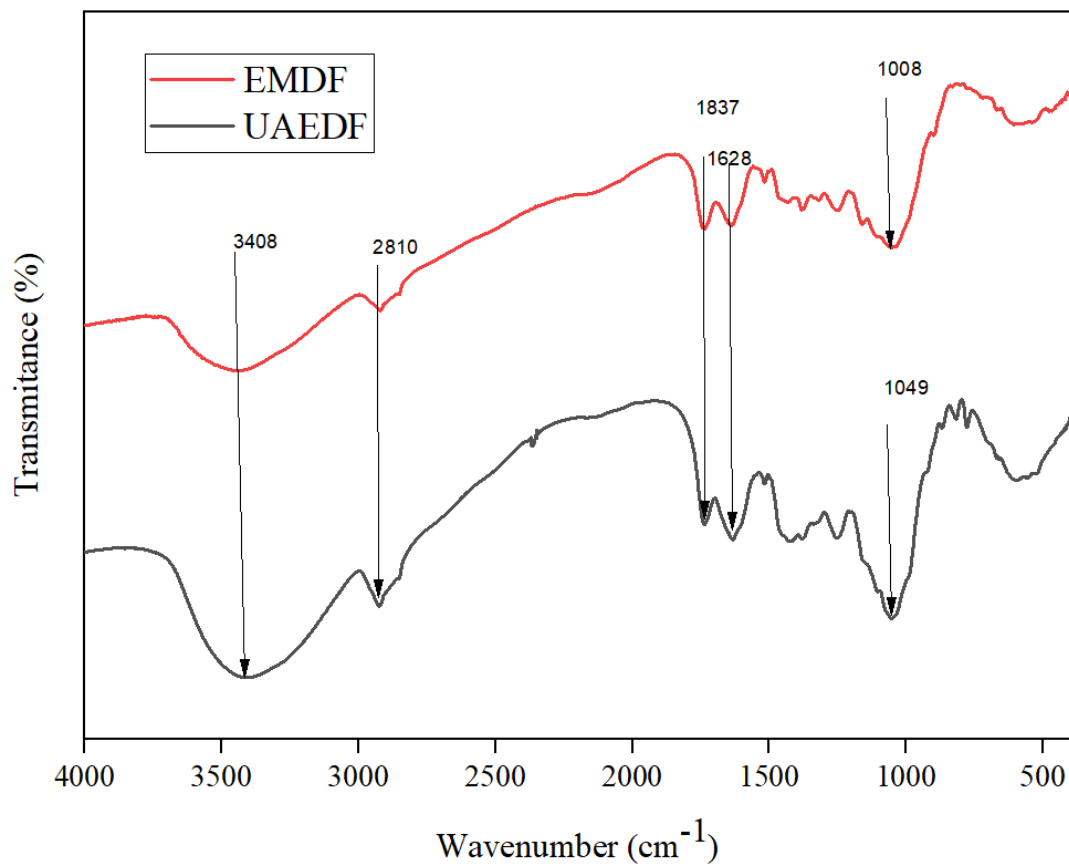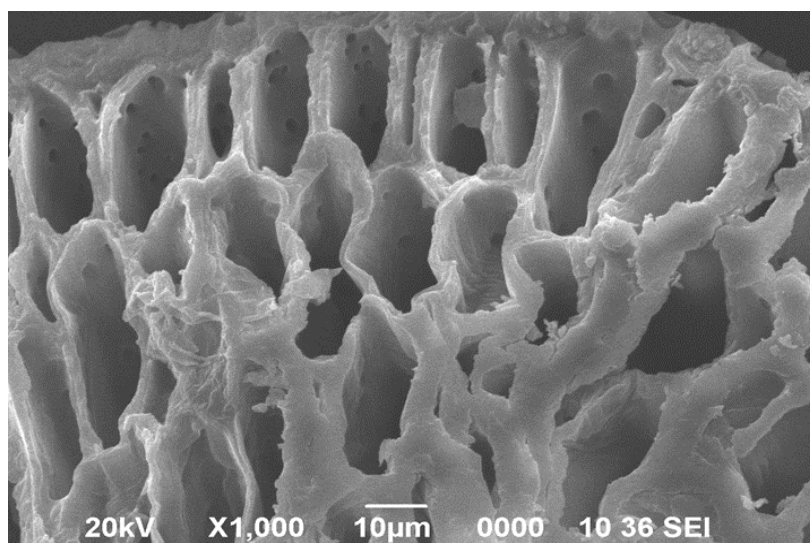

Fig.3.1: FTIR of Ultrasonic-assisted extracted dietary fiber (UAEDF), and Enzyme modified dietary fiber (EMDF)

The cellulase/xylanase treatment raised this absorbance peak, indicating improved water absorption capacity within the dietary fibre matrix made of pineapple waste. It is believed that the "fingerprint" of carbohydrates is represented by peaks in the 800–1200 cm⁻¹ range. At wavelengths of 1050 cm⁻¹ in EMDF and 1157 cm⁻¹ in UAEDF, the stretching of C–O groups in functional acid and ether groups inside aromatic C–O–C was the primary cause of significant absorption bands. These peaks dramatically decreased upon enzymatic treatment of the dietary

fibre, indicating degradation into oligosaccharides. In summary, cellulase/xylanase modification disrupted the amorphous regions of dietary fibres altered by enzymes, broken polymers, and disrupted the intermolecular hydrogen connections between hemicellulose and cellulose. The capacity to hold water would have been enhanced since there were more hydrophilic groups and binding sites as a result.

3.3.2 SEM of modified dietary fibre:

The morphological characteristics of the DF are depicted in Fig. 3.2. With a magnification increase of 1000-fold, particle size remained constant at 10 μm, revealing notable disparities among pineapple waste dried powder, ultrasound-assisted extracted dietary fiber, conventionally extracted dietary fiber, and enzyme-modified dietary fibers. A scanning electron microscope (SEM) was used to see these morphological changes. The intact tissue structure and compact layer of the UAEDF were accompanied by a smooth surface and thick texture devoid of holes. On the other hand, the enzyme-modified dietary fiber (EMDF) surface showed more cracks and was uneven and rough. The interior structure became visible as a result of the disruption of the dense lamellar structure, which left a loose and porous arrangement. The EMDF surface area was greatly increased by this structural alteration, which improved its solubility and water-holding capacity (Yan et al., 2023). More polar and non-polar groups are exposed by this structure, which enhances water hydration and adsorption ability (Benitez et al., 2019). According to Ma et al. (2022), dietary fiber's monosaccharide content and microstructure can be changed by enzyme treatment, enabling greater interaction with molecules such as glucose, cholesterol, and other substances and improving the physiological properties of dietary fiber.
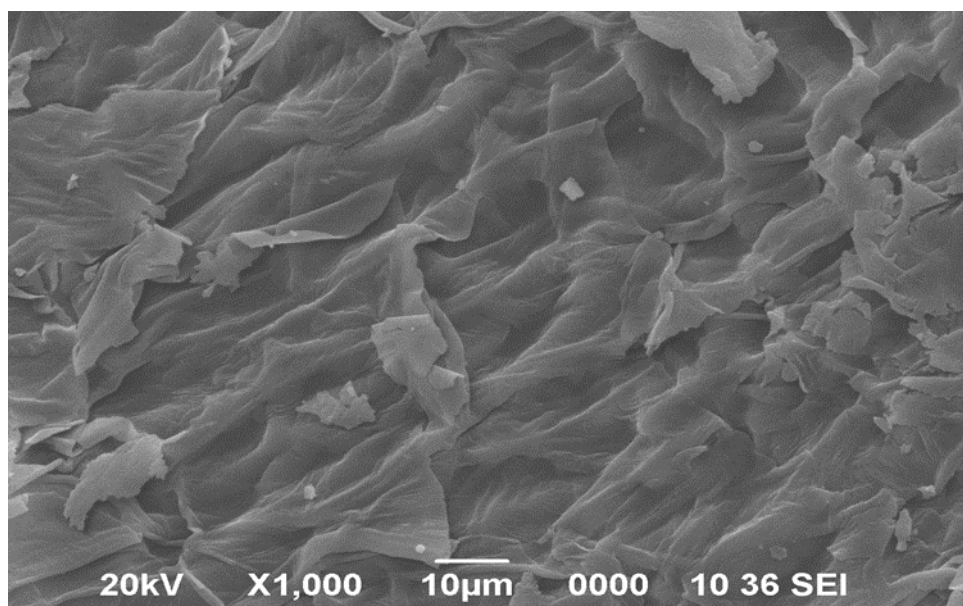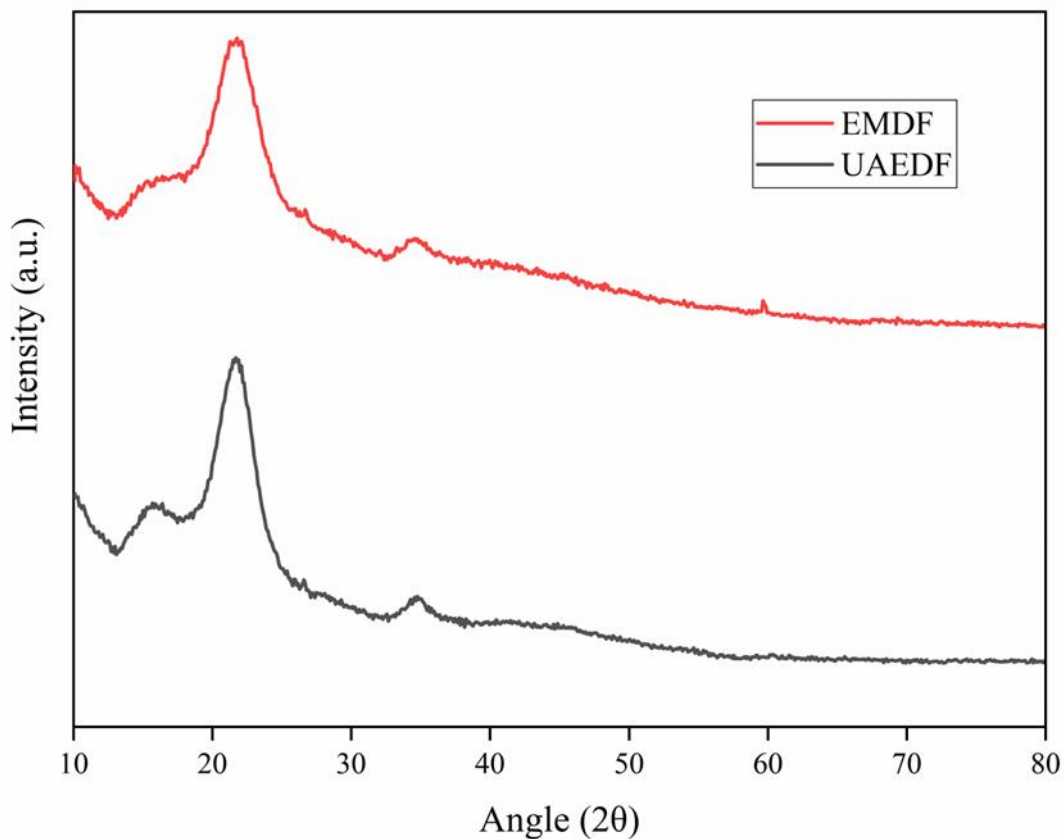
Fig.3.2: Microstructure of (a) Ultrasonic-assisted extracted dietary fiber (UAEDF), and (b) Enzyme modified dietary fiber (EMDF)

3.3.3 XRD of modified dietary fibre:

The X-ray diffraction (XRD) patterns of the dietary fibers are illustrated in Fig. 3.3. An intact crystal surface and elongated crystalline lines are indicative of the existence of crystalline cellulose, as indicated by the typical spike in the XRD (Osorio et al., 2021). The cellulose crystal structure shows two different diffraction peaks at $2\theta = 18.11°$ and $26.17°$ in both EMDF and UAEDF, confirming the presence of crystalline cellulose regions. Furthermore, irregular weak peaks detected at $2\theta = 28.39°$, $31.14°$, and $35.13°$ might result from enzymatic hydrolysis-induced cellulose degeneration in certain dietary fibre segments, implying that peaks at 15–16 Å and 20–21 Å might be ascribed to cellulose crystals. Non-crystalline cellulose, hemicellulose, and lignin make up the amorphous areas of cellulose, which are kept amorphous by hydrogen bonding interactions and van der Waals forces between neighbouring molecules (Liao et al., 2020).
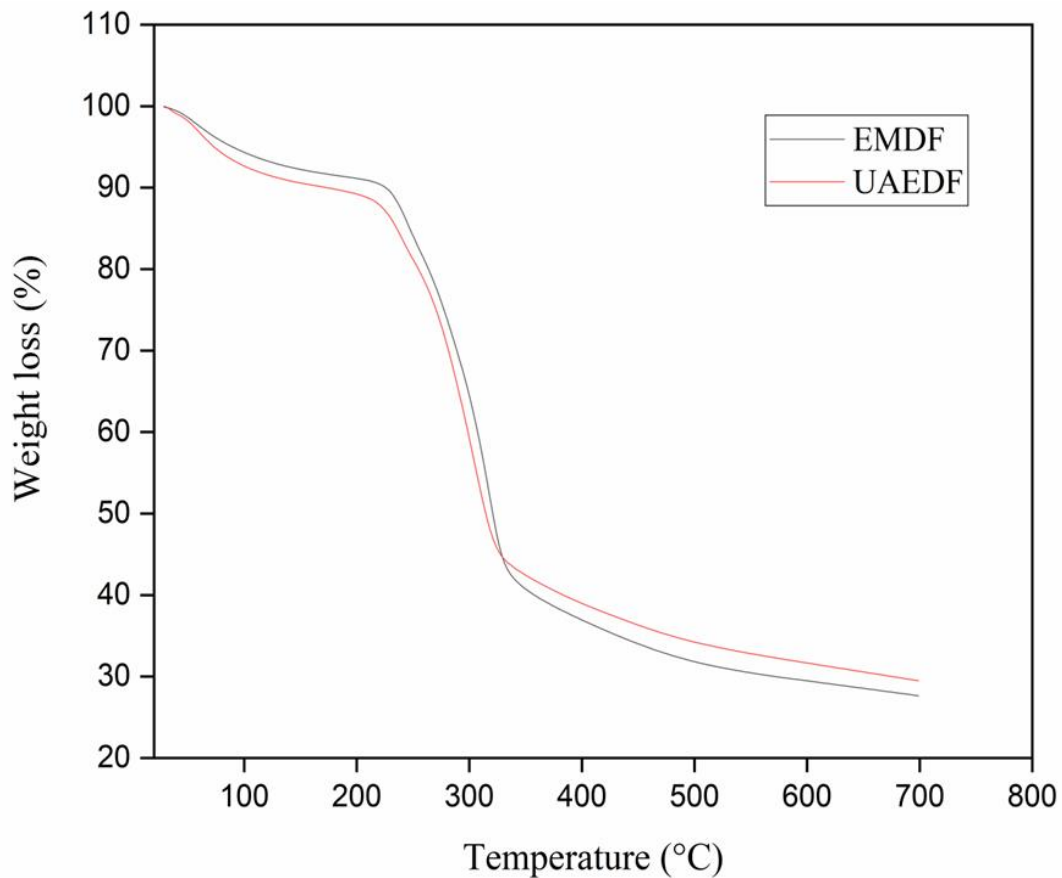
Fig.3.3: XRD of Ultrasonic-assisted extracted dietary fiber (UAEDF), and Enzyme modified dietary fiber (EMDF)

The peak position and width stayed mostly unaltered, suggesting that the cellulose crystalline region's ordered structure in the dietary fibre treated by enzymes did not change. Enzymatic hydrolysis may be the cause of the modified dietary fiber's somewhat decreased peak intensity. Additionally, the appearance of multiple new high-intensity peaks in the EMDF indicates a strong impact from enzymatic modification aided by ultrasonication on the production of new crystals.

3.3.4 TGA of modified dietary fibre:

Thermogravimetric Analysis (TGA) was employed to assess the thermal characteristics and stability of ultrasound-assisted dietary fiber (UAEDF), and enzyme-modified dietary fiber (EMDF) under a nitrogen atmosphere. As the temperature ranged from 30.0 to 650 ºC, three distinct weight loss peaks were observed in all samples (Fig. 3.4). The initial weight loss stage exhibited relatively slow weight changes for both UAEDF and EMDF. The weight loss peak

for EMDF occurred at 56.5 ºC, with a value of 2.80%, due to the evaporation of crystal and adsorbed water in polysaccharides (Cheng et al., 2022).

Fig.3.4: TGA of Ultrasonic-assisted extracted dietary fiber (UAEDF), and Enzyme modified dietary fiber (EMDF)

During the second stage, all of the dietary fibre samples showed a noticeable and quick weight loss peak between 200.0 and 400.0ºC. The UAEDF peak shape was sharper than that of EMDF, which could be attributed to the thermal breakdown of hemicellulose polysaccharides, which is similar to the pre-carbonization process of cellulose in dietary fibres (Wang et al; 2022). For UAEDF, and EMDF, the corresponding thermal breakdown temperatures were 299.91, 326.5, and 357.5 ºC. The modified dietary fibre showed a weight loss peak during the third stage at 418.5 ºC, with a weight loss rate of 18.43%. Below 200.0 ºC, the dietary fibres remained generally steady. At this juncture, EMDF displayed distinct thermal properties from UAEDF, suggesting potential thermal cracking reactions of certain components in the modified dietary fiber at temperatures ranging from 500.0 to 700 ºC.

3.3.5 Functional properties 3.3.4 TGA of modified dietary fibre:

The water-holding capacity (WHC), swelling capacity (SC), and oil-holding capacity (OHC) of pineapple waste extracted dietary fibers are crucial for their applications in food. Table 3.2 presents the WHC, SC, and OHC values of dietary fibers post-enzymatic modification. Both ultrasound-assisted extracted dietary fiber (UAEDF) and enzyme-modified dietary fiber (EMDF) exhibited significantly increased WHC compared to raw material PDP ($p < 0.05$), with EMDF showing a WHC of 15.78 g/g. WHC lowers syneresis, which lowers food quality. It is regulated by physicochemical characteristics and environmental factors such as ionic strength and structural morphology, particularly in functional foods. Enzymatic treatment exposes hydrophilic groups and alters spatial structures and ratios between crystalline and amorphous regions, facilitating water molecule penetration into fiber structures (Zhang et al., 2023).

OHC is vital for evaluating dietary fiber quality, as it prevents oil loss during food processing and reduces oil absorption during human digestion. Both UAEDF and EMDF showed significantly increased OHC compared to PDP ($p < 0.05$). Differences in dietary fiber composition and structure from various raw materials lead to variations in oil retention abilities. Enzymatic treatment, as reported by Fan et al. (2020), alters hydrophilic groups and fiber structure, enhancing OHC, as demonstrated in this study. The highest OHC of EMDF was 7.96 g/g, attributed to enzymatic modification breaking cell walls, resulting in looser fiber structure and increased porosity (Ma & Mu, 2016).

The SC of EMDF after enzymatic treatment was 8.68 mL/g, consistent with studies linking SC and WHC to the existence of hydrophilic groups in dietary fiber structures. These studies align with FTIR findings.

Emulsion activity (EA) values for UAEDF and EMDF were significantly reduced compared to PDP ($p < 0.05$), with stability index values below 50% (Table 3.2), indicating poor emulsifying properties. This is consistent with previous reports of low EA values for dietary fiber (Kalla-Bertholdt et l., 2021). Lower protein content in UAEDF and EMDF may contribute to these low EA values.

The cation exchange capacity (CEC) of UAEDF and EMDF was significantly higher than PDP ($p < 0.05$), with EMDF showing a higher CEC than UAEDF ($p < 0.05$), reaching 7.83 mM/g. Enzymatic hydrolysis breaks covalent bonds of DF molecules, imperiling more carboxyl and

hydroxyl groups conducive to cation exchange capacity, thus improving CEC of dietary fibers (He et al., 2020).

Table 3.2: Functional properties of PDP, UAEDF and EMDF

Functional properties	Pineapple waste dried powder (PDP)		Ultrasonic-assisted extraction dietary fiber (UAEDF)		Enzyme modification dietary fiber (EMDF)	
WHC(g/g)	8.64±0.09[a]		12.72±0.16[a]		15.78±0.01[a]	
OHC (g/g)	3.53±0.012[a]		5.22±0.10[a]		7.96±0.05[a]	
SC (mL/g)	4.23±0.10[a]		7.38±0.28[a]		8.68 ± 0.23[b]	
EA (%)	31.89±1.88[bc]		21.89±1.42[c]		18.98±0.46[d]	
ES (%)	17.8±1.3[b]		29.5 ± 2.1d		31.20±0.26[bc]	
CEC (mM/g)	3.98±0.14[a]		6.34±0.62[b]		7.83±0.28[bc]	
CAC (mg/g)	**pH 2.0**	**pH 7.0**	**pH 2.0**	**pH 7.0**	**pH 2.0**	**pH 7.0**
	6.38	12.12	9.79	16.56	12.71	22.67

The values presented are expressed as mean ± SD; with a sample size of n=3. When means within the same row are marked with different superscript letters, it indicates a significant difference at p<0.05.

*WHC - Water Holding Capacity; OHC - Oil Holding Capacity; SC - Swelling Capacity; EA - Emulsion Activity; ES - Emulsion Stability; CEC - Cation Exchange Capacity; CAC - Cholesterol Absorption Capacity

In vitro cholesterol adsorption capacity (CAC) assays conducted at pH 2.0 and pH 7.0 revealed significant cholesterol adsorption affinity for both UAEDF and EMDF. The CAC of both UAEDF and EMDF at pH 7.0 was significantly higher than at pH 2.0 ($p < 0.05$), indicating easier cholesterol absorption in the small intestine than in the gastric tract. EMDF exhibited a CAC of 28.67 mg/g at pH 7.0 and 12.71 mg/g at pH 2.0, attributed to carboxyl, hydroxy, and amidogen groups in macromolecules conferring weak acidic cation exchange resin properties to dietary fiber, regulating blood pressure through changes in ion concentration, REDOX potential, and intracellular osmotic pressure (Zheng et al., 2021).

3.3.6 Hypoglycaemic activity:

3.3.6.1 GAC of modified dietary fibre:

The hypoglycemic activity of dietary fiber is a critical characteristic for assessing glucose adsorption capacity (GAC). In vitro determination of GAC can provide insights into the impact of dietary fibers on blood glucose management (Liu et al., 2018). As shown in Table 3.3, both ultrasound-assisted extracted dietary fiber (UAEDF) and enzyme-modified dietary fiber (EMDF) exhibited significantly higher GAC at different glucose concentrations in dialysate of 10, 50, 100, and 200mM ($p < 0.05$), with EMDF reaching 120.52 mg/g. The enhanced GAC of EMDF may stem from the elevated soluble dietary fiber (SDF) content in pineapple waste extracted dietary fiber. SDF can influence viscosity, thereby delaying glucose diffusion.

Table 3.3: GAC (mmol/g) of UAEDF and EMDF

Dietary fiber	Glucose concentration in dialysate (mM)			
	10	50	100	200
UAEDF	23.28±0.08	42.28±0.04	61.98±0.33	87.18±0.12
EMDF	31.78±0.08	59.89±0.45	109.38±0.29	120.52±0.13

Furthermore, extraction and modification processes could loosen the surface structure of dietary fiber, increasing lacunae and surface area. These structural modifications effectively intercepted glucose molecules, thereby influencing glucose absorption and transportation (Geng et al., 2023). EMDF demonstrated the highest GAC, suggesting that enzyme-modified dietary fiber holds greater potential for blood glucose management applications.

3.3.6.2 GDRI of modified dietary fibre:

Glucose diffusion retardation index (GDRI) was evaluated at different time intervals (30, 60, 120, and 180 minutes) for pineapple waste-extracted dietary fiber (UAEDF, EMDF) and the control group. In the control, dialysate glucose content steadily rose from 20.12 to 90.16 μmol within the observed time frame (30 to 180 minutes). Conversely, dietary fiber extracted from pineapple waste through ultrasound and enzyme modification exhibited a slower increase in dialysate glucose content compared to the control. Specifically, glucose content increased from 12.86 to 71.23 and 15.16 to 78.93 for UAEDF and EMDF, respectively, over the 30 to 180-minute period (Table 3.4). GDRI serves as a valuable in vitro metric, indicating the fiber's capacity to delay glucose absorption in the gastrointestinal tract, correlating with fiber

properties such as structural characteristics, surface properties, soluble dietary fiber content, and uronic acid content. The presence of soluble dietary fiber in bracts further influenced the retardation of glucose diffusion. Hence, GDRI values for UAEDF and EMDF were notably higher than that of cellulose, primarily composed of insoluble dietary fiber. These values exceed those reported for various fiber-rich byproducts such as artichoke fiber (27%), mango peel (21%), and asparagus fiber (18-47%), as documented by Hasnaoui et al. (2014). This substantial difference may be attributed to the antihyperglycemic properties of banana flower. Studies have shown that banana flower (Musa sp. var. elakki bale) exhibits antidiabetic effects, inhibiting the formation of Advanced Glycation End Products (AGEs) in streptozotocin-induced diabetic rats (Begum and Deka, 2019). These findings suggest that dietary fiber derived from pineapple waste holds promise in regulating postprandial blood glucose levels and could be utilized in formulations for antidiabetic therapies. Additionally, it could serve as a low-calorie, fiber-rich ingredient for dietetic snacks.

Table 3.4: Glucose dialysis retardation index (GDRI) of ultrasound-assisted extracted dietary fiber (UAEDF) and enzyme modified dietary fiber (EMDF)

Glucose in dialysate (µmol)				
Sample	30 min	60min	120 min	180min
UAE DF	12.86±0.25	21.75±0.15	30.32±0.05	71.23±0.18
EDF	15.16±0.15	26.12±0.35	41.21±0.15	78.93±0.25
Control	20.12±0.05	29.25±0.03	54.23±0.50	90.16±0.35

3.4 Conclusion:

Enzymatic modification of dietary fiber derived from pineapple waste resulted in increased soluble fiber content and improved structural characteristics, rendering it a promising functional ingredient for food applications. Future research endeavors could explore the rheological and textural attributes of the modified dietary fiber when incorporated into food formulations. The comprehensive findings revealed that enzyme-modified dietary fiber (EMDF) obtained from pineapple waste exhibited enhanced water-holding capacity (WHC), swelling capacity (SC), oil-holding capacity (OHC), cation exchange capacity (CEC), cholesterol adsorption capacity (CAC), and demonstrated hypolipidemic effects. The elevated WHC, SC, and OHC of EMDF can be attributed to the exposure of hydrophilic groups.

Moreover, the higher CEC of EMDF is indicative of its favorable physicochemical properties. The increased CAC of EMDF is attributed to its structural alterations, expanded surface area, and higher porosity as demonstrated by scanning electron microscopy (SEM).

In summary, enzymatic modification of pineapple waste dietary fiber offers the potential to enhance its functional attributes and broaden its application in food products. This research contributes to the sustainable utilization of pineapple waste and the development of value-added ingredients for the food industry.

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