CHAPTER-3

Effect of extrusion and enzyme modification on functional and structural properties of pea peel (*Pisum sativum* L.) insoluble dietary fiber and its effect on yogurt rheology

3.1 Introduction

Pea (*Pisum sativum* L.) is a major crop that contains bioactive chemicals that are beneficial to consumers' health. It is rich in dietary fibers, proteins, phenolic compounds and essential amino acids [7, 8]. A huge amount of pea by-products is generated in various food processing industries, which are discarded as waste and have not generally gained much attention [11]. In agro-industries, the greatest constraint in discarding peels is economic flexibility. However, pea peels have been reputed to be a startling source of dietary fiber [18] and this waste utilization can reduce the carbon footprint. Hence the intention of this study is to utilize the pea peel dietary fiber to emphasize its nutritional values for using as potential additives in food industries.

Various studies highlighted that dietary fiber could be delineated as the non-starch polysaccharide collections that have the capability to resist the enzymatic digestion in the small intestine. However, partial or complete fermentation occurs in the large intestine digestion process [27]. On a solubility basis, dietary fiber (DF) could be fragmented into two sub-categories viz., soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) [30]. Based on the structural differences and degree of polymerization, SDF and IDF possess different physiological functions [10, 9]. SDF exhibited a role to reduce plasma cholesterol and glycemic response along with immune-modulatory activity. It also helps to prevent colorectal cancer and also reduce the risk related to angiocardiopathy [10]. IDF has shown a greater affinity towards the bulking faecal, reducing gastrointestinal transit time, inhibiting pancreatic lipase activity, and preventing diseases like colonic cancer and obesity [27, 29]. Due to its good processing and functional attributes and potential use towards health and food industries, it has drawn consumers' attention for investigation of the different sources of DF.

Based on the health impacts, SDF plays a substantial role compared to IDF, but the IDF constituent in most of the plant-based products is about 60-80%. Therefore the modification of IDF is of paramount important to enhance the functionality at chemical, physical, biological, and molecular levels.

There are various techniques used in order to modify the DF constituents viz., chemical (e.g., alkali and acid method), physical (e.g., ultrahigh pressure, extrusion technology and ultrafine pulverization) and biotechnological (e.g., enzymatic method) that could break the glycosidic bonds of IDF by lowering the degree of polymerization [10, 12]. The modification leads to the breakdown of pectic polysaccharides or the degradation of partial cellulose and hemicellulose into simple saccharide structures. Ash and pectin contents are also greatly reduced by the modification [17]. The IDF microstructure linked together with bloc and platy shape where it possesses a dense structure having no pores. The modification removed the protein around the DF showing a spongy structure having more porosity exposed to hydroxyl groups [13]. Biological and physical methods are highly recommended to modify DF where the enzymatic modification could enhance the extraction yield and considered to be an effective method for reducing the particle size because of their high efficiency and high specificity [26, 29]. In the native state, fibers possess poor functionality because of cellulose and hemicelluloses that offer a compact polymeric structure to IDF, where the modification improves its nutritional qualities by loosening the tight structure, increasing porosity, and facilitating partial degradation [26]. Various processing conditions alter the composition and micro profile of IDF which eventually enhances the functional and physicochemical properties [30]. It was reported that the SDF content could be increased by extrusion as well as by enzymatic hydrolysis [31, 1]. Carrot pomace IDF was modified using a complex enzymatic and other thermal methods where SDF content increased up to 15.07% [30]. The thermomechanical treatment such as the extrusion method was also able to modify apple pomace DF by 12 to 16 g/100 g dry matter upon disruption of the rigid macromolecular structure of the cell wall [23]. Wheat bran DF modification alters the IDF to SDF fragments significantly with its improved water retention and water-solubility properties, using enzymatic and extrusion processes [1]. The modification of citrus peel has also been reported [24] and compared among the enzymatic and physical viz., planetary ball milling treatment. However, for pea peel IDF modification, the study has not been done before. Therefore, the effect of different modifications in

physicochemical, functional, and structural properties of pea peel dietary fiber are necessary to explore its suitable application. In the backdrop of this, the present study primarily focuses on the use of extrusion cooking and enzymatic modifications of pea peel IDF to improve the structural, functional, and physicochemical characteristics and further the impact of these properties of the modified IDF was examined on the flow behaviour of yogurt.

3.1. Materials and methods

3.1.1. Materials

Green peas (*Pisum sativum* L.) were procured from Tezpur local market, Assam, India. The green pea peels (GPP) were separated and dried in a tray dryer (Model # BDI-51, Labotech, Make # Delhi, India) at 55^0C for 12 h followed by grinding and de-oiling process using 95% ethanol. The enzymes viz., cellulase (300 U/g), xylanase (2500 U/g), and all other chemicals were of high purity analytical grade (Sigma-Aldrich Co).

3.1.2. Ultrasound-assisted alkaline extraction

NaOH concentration 1.2%, extraction time 30 min, solid to liquid ratio 1: 30 (w/v), and ultrasonic amplitude 30% were used as independent parameters. Extraction was performed under controlled ultrasound-assisted alkaline conditions using a probe-type ultrasonicator (Model # Q700-220 Digital Sonicator, Qsonica LLC, Make # USA). After extraction, the suspension was centrifuged at 5000x*g* for 20 min where the residue was collected and washed once with distilled water followed by drying in the oven at 60°C until constant weight was attained [12].

3.1.3. Modification of IDF

3.2.3.1. Enzymatic modification

The IDF (2% w/v) was suspended to 60 ml of disodium hydrogen phosphate citrate buffer and pH was calibrated to 5.0. Cellulase (90U/g, 65U/g, and 21U/g) and xylanase (21U/g, 65U/g, and 90U/g) with varied concentrations were added to the sample and the mixture was incubated in the water bath at 50^0C for 120 min [30, 29]. After enzymolysis, 4 times 95% ethanol was added to the solution and then cooled down to room temperature (25 ± 1^0C). The residue was filtered using Whatman no.1 and dried in a

hot air oven at 50^0C followed by grinding and sieving through a 250 µm and stored at -20^0C until further use and coded as M1 (90U/g cellulase: 21U/g xylanase), M2 (65U/g cellulase: 65U/g xylanase), and M3 (21U/g cellulase: 90U/g xylanase).

3.2.3.2. Extrusion modification

Pea peel IDF was modified using a twin-screw extruder as per method described by Bader Ul Ain et al. [2] where the twin-screw extruder had 25 mm co-rotating screws (Model # FUE-1F, Flytech Engineering, Make # Chennai, India). Preliminary trials were done to ensure the suitable condition for IDF modification. The barrel temperature was varied at 130^0C, 140^0C, and 170^0C, feed moisture content from 20% to 40% and screw speed was 70 rpm. Extrudate was gently dried and stored at 4°C for further analysis and coded E1 (20%, 130°C, 70 rpm), E2 (30%, 140°C, 70 rpm), and E3 (40%, 170°C, 70 rpm).

3.1.4. Preparation of yogurt supplemented with modified IDF

A set type of yogurt was prepared with *Streptococcus thermophilus* and *Lactobacillus. delbrueckii* ssp. *bulgaricus* as prescribed by [22]. The yogurt was fortified with 2% modified IDF. The fortified milk was homogenized and boiled at 85^0C for 30 min and then cooled till 45^0C was attained. Thereafter 1% (v/v) of *S. thermophilus* and *L. bulgaricus* monoculture was aseptically inoculated. After 5 h of incubation, the yogurt was prepared and cooled immediately and stored at 4^0C for further use. The control sample was prepared with the incorporation of unmodified dietary fiber and coded as 'YC'.

3.1.5. Scanning electron microscopy

Morphological properties of the modified IDF were examined by scanning electron microscope (Model # JSM-6390LV, Make # JEOL, Japan) at 20 kV. Dried samples were kept on a specimen holder using a copper plate which was coated by a thin gold film followed by ion sputtering method and the images of samples were taken at 1000 and 1500X magnifications, respectively.

3.1.6. FT-IR

The FT-IR spectra of the modified IDF was analysed by an FT-IR spectrometer (Model # Nicolet Instruments 410 FTIR, Make # Thermo Scientific, USA) at 400−4,000 cm^{-1}

wavenumbers at the resolution of 4 cm^{-1}.

3.1.7. Crystallinity

The crystalline structure of the modified IDF was determined by X-ray diffractometer (XRD) (Model# Bruker AXS, Make # Germany) [32]. The pattern was recorded with a scanning speed of 1^0 min^{-1} and incident current of 40 mA over a diffraction angle (2θ) range of 5-70.

3.1.8. Brunauer-Emmett-Teller surface area

The Brunauer-Emmett-Teller (BET) surface area of the modified IDF was analysed by nitrogen gas adsorption at -196^0C using a BET analyser (Model # ASiQC0000-4, Make # Quantachrome, USA). Initially, the sample was degassed under vacuum conditions at 100^0C for at least 8 h. Over a relative pressure (P/P_o) range from approximately 10.5 to 0.995, nitrogen adsorption isotherms were measured. The determination of the BET surface area of the modified IDF samples was done using the standard BET equation having relative pressure from 0.06 to 0.3.

3.1.9. Thermal properties

Thermal properties were performed for modified IDF using differential scanning calorimeter (DSC) (Model # Q200 TA Instruments, Make # DE, USA) where 5 mg samples were taken and sealed in aluminium pans under a nitrogen environment at a heating rate of 5°C min^{-1} from 20°C to 300°C. An empty sealed pan was taken as the reference. Peak transition temperature (T_p), onset transition temperature (T_0), and final transition temperature (T_c) were measured.

3.1.10. Thermal decomposition

The thermal decomposition behaviour of modified dietary fiber powder was analyzed by thermogravimetric analysis (TGA), (Model # NETZCH TG 209F1, Make # Libra, Germany). The heating range used was from 25 to 600^0C at a rate of 10^0C/min under a nitrogen atmosphere. The degradation temperature (T_d) and weight loss were evaluated.

3.1.11. Water retention capacity

Water retention capacity (WRC) was performed following the method described by Yu et al. [30]. IDF (0.2 g) was suspended in 6 mL distilled water for 18 h at room

temperature (25±1^{0}C) and then centrifuged at 5000xg for 15 min. At 105^{0}C, the resulting residue weight was recorded before and after drying to a constant weight. WRC was calculated by the following equation:

$$WRC = (M_f - M_d)/M_d \quad \ldots(1)$$

Where, M_f is the weight of the fresh residue (g) and M_d is the weight of the dry residue (g).

3.1.12. Swelling capacity

For the measurement of swelling capacity (SC) the IDFs (1g) was hydrated in 15 mL distilled water in a graduated test tube for 24 h at 25^{0}C [30]. The bubbles inside the test tubes were removed by gentle stirring. The bed volume was recorded and the SC was calculated by the following equation:

$$SC\ (\frac{mL}{g}) = (V_1 - V_0)/W_0 \quad \ldots(2)$$

Where, V_1 is the volume of the hydrated DF (mL), V_0 is the volume of the IDF prior to hydration (mL), and W_0 is the weight of the IDF prior to hydration (g).

3.1.13. Oil absorption capacity

Oil retention capacity (OAC) was performed in a similar way to WRC wherein the water was replaced with soybean oil. Oil retention capacity was measured as g soybean oil absorbed per g of dry sample [20].

3.1.14. Cation exchange capacity

The cation exchange capacity (CEC) of the modified IDF was calculated according to Zhou et al. [32]. Samples (1 g) were hydrated in 50 mL of 0.1 mol/L hydrochloric acid. The suspension was vacuum filtered after the continuous stirring of 24 h. To retain the pH of the supernatant above 4, the residue was washed thoroughly for multiple times with distilled water. Once the residue became acidic, it was suspended in 100 mL of 15% sodium chloride together with a blank containing residue with distilled water. After centrifugation, the supernatant was titrated with 0.1 mol/L sodium hydroxide and the CEC was calculated as follows:

$$CEC\ (\frac{mmol}{g}) = 0.1 \times (V_1 - V_0)/M_d \quad \ldots(3)$$

Where, V_1 is the titrated volume of sodium hydroxide of the sample (mL), V_0 is the titrated volume of sodium hydroxide of the blank (mL), 0.1 is the concentration of sodium hydroxide (mol/L) and M_d is the dry weight of the sample (g).

3.1.15. Glucose *adsorption capacity*

The glucose adsorption capacity (GAC) of the modified samples was calculated as described by Qiao et al. [20]. Briefly, 1 g sample was mixed with 100 mL glucose solution (100 mmol/L) and incubated at 37^0C in a water bath at 150 rpm for 6 h. After centrifugation at 4000 g for 15 min, the glucose content in the supernatant was measured using a glucose assay kit (GAGO20-KT, Sigma); the GAC was expressed as follows (Eq. 4):

$$\mathrm{GAC}\left(\frac{\mu\mathrm{mol}}{\mathrm{g}}\right) = (C_0 \times V_0 - C_1 \times V_1)/\mathrm{m} \quad \ldots(4)$$

Where, C_0 is the original glucose concentration (mM), C_1 is the glucose concentration in the supernatant (mM), V_0 is the original volume (mL), V_1 is the supernatant volume (mL), and m is the weight of the fiber (g).

3.1.16. Glucose dialysis retardation index

The glucose dialysis retardation index (GDRI) was calculated according to the method described by Lin et al. [13]. Briefly, 0.5 g of each sample was thoroughly hydrated in 15 mL of 0.2% glucose solution by stirring for 1 h and transferred to a pre-hydrated dialysis bag (Model # 8,000 MWCO; Make # Sigma-Aldrich, USA). The control bag contained glucose but no fiber and other bags with fibers were placed in a beaker containing 200 mL of distilled water and shaken (160 rpm) at 37^0C for 1 h. An aliquot of the dialysate was collected every 15 min to measure the glucose concentration. GDRI was calculated using Eq. 5:

$$\mathrm{GDRI}\ (\%) = \left(1 - \frac{(A_1 - A_2)}{A_3}\right) \times 100 \quad \ldots(5)$$

Where, A_1 is the total glucose diffusion from the sample, A_2 is the total glucose diffusion from the conditional control and A_3 is the total glucose diffusion from control [13].

3.1.17. Color of the yogurt

The color parameters (L*, a*, and b*) of yogurt samples fortified with the modified IDF were measured using a colorimeter (Hunter Color-Lab Ultrascan Vis.). The chroma (C*, brightness) and hue angle were calculated using Eq. (6) and Eq (7), respectively.

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad \ldots(6)$$

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \ldots(7)$$

Different values of hue angle represent various colors: 0^0 = red-purple, 90^0 = yellow, 180^0 = bluish-green and 270^0 = blue.

3.1.18. Oscillatory dynamic rheology of yogurt supplemented with modified IDF

The rheological properties of yogurt incorporated with the modified IDF were measured using a controlled-stress rheometer (Model # Antron Paar, Physical MCR 301, PP 50). A constant strain of 0.5% was employed over the linear viscoelastic regime. The sample was placed with 1 mm gap in between the plates followed by 10 min rest before testing. The frequency sweep tests between 10 and 100 rad/s were conducted at 25^0C, yielding 100 data sets of storage modulus (G′) in 22 minutes and each experiment were examined thrice

3.1.19. Statistical analysis

Data were analyzed using SPSS version 25.0 and expressed as triplicate determinations of means±standard deviations. The analysis of variance (ANOVA) and Tukey's comparison test were used to detect significant differences ($p < 0.05$).

3.2. Results and discussion

3.2.1. Microstructure

The unmodified IDF exhibited a typical clean honeycomb network with no fibrous structure (Fig 3.1 C). The extrusion cooking at three different temperatures brought a noticeable surface structure modification of IDF (Fig 3.1 E1, E2, and E3). The surface structure has been modified appreciably due to high shear in presence of high temperature during extrusion treatment. Moreover, at increased extrusion temperature there was a reduction in the molecular weight of larger molecules, resulting in straight smaller fragments as observed in (Fig 3.1 E3) which probably destroys the partial structure of fibers. Consequently, more hydroxyl groups are generated making them more fibrous and rough. However, a different microstructure was observed for cellulase and xylanase modified IDF as shown in Fig 3.1. M1, M2, and M3. The number of cavities/spaces was distinctly visible on the surface of the enzyme treated IDF due to the specificity of enzymes, whereas no such cavities/spaces were visible in the unmodified sample. The breakdown of glycosidic linkage mediated by cellulase, and xylanase may result in a loosely structured honeycomb structure and deeper voids on the surface of enzyme-treated IDF [32, 26].

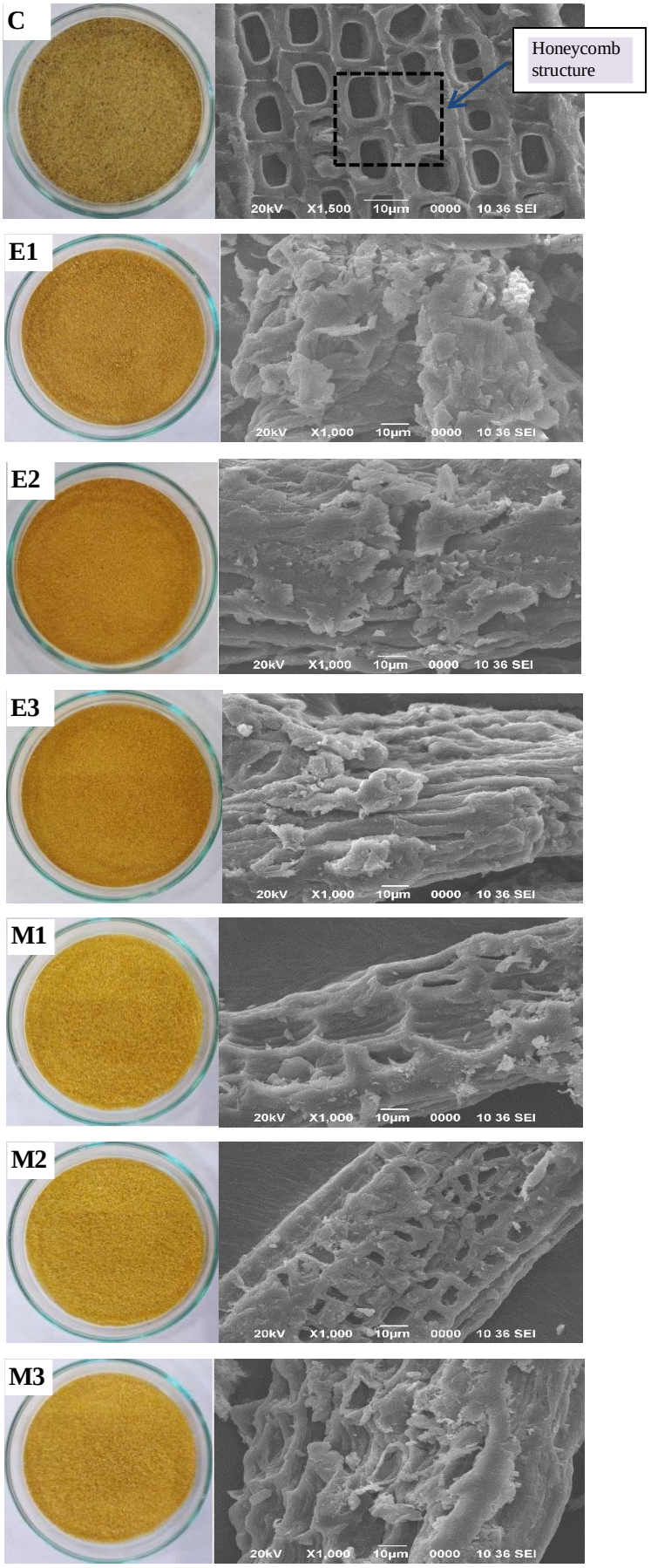

Fig 3.1 Scanning electron microscopy images of unmodified (C) and modified insoluble dietary fiber of pea peel (*Pisum sativum L.*) by extrusion (E1, E2, E3) and enzymes (M1, M2, M3) treatment

3.2.2. FT-IR analysis

FT-IR spectroscopy is normally used to indicate the functional group compositions and is found sensitive to molecular structure differences [17]. The modified IDF showed a typical polysaccharide absorption band in the range of 3300 to 3550 cm^{-1} (Fig 3.2). The O-H tensile vibration in phenol, alcohol, and other hydroxyl groups comprising cellulose, hemicellulose, or lignin [15, 6] was observed. All the samples showed peaks at 2907 cm^{-1} depicting C-H stretching of the methyl or methylene group of cellulose. The slight absorption spectra displayed by all the samples at 2907 cm^{-1} indicated an asymmetric C-H methylene vibration. Increased intensity at 1650 cm^{-1} showed bending of the carbonyl group. There was a distinct peak formed at 1415 cm^{-1} by extruded modified samples (E1, E2, and E3) which represents $–CH_3$ stretching vibration indicating the presence of carboxymethyl group [26]. The prominent peak at 1025 cm^{-1} in the fingerprint region of carbohydrate formed by extrusion modified polysaccharide is due to linkage formed by lignin and hemicellulose and was slightly decreased by enzymolysis of the modified sample (M1, M2, and M3). The spectrum at 1514 cm^{-1} appeared for extrusion modification, represented the C-C bond whereas other peaks at around 890 cm^{-1} indicated acrylate groups [5]. Collectively, modification by extrusion and enzyme method lysed the intermolecular region of hydrogen bond (cellulose and hemicellulose), destroyed the amorphous region, leading to enhancement of hydrophilic groups and which could increase the water holding capacity [6].

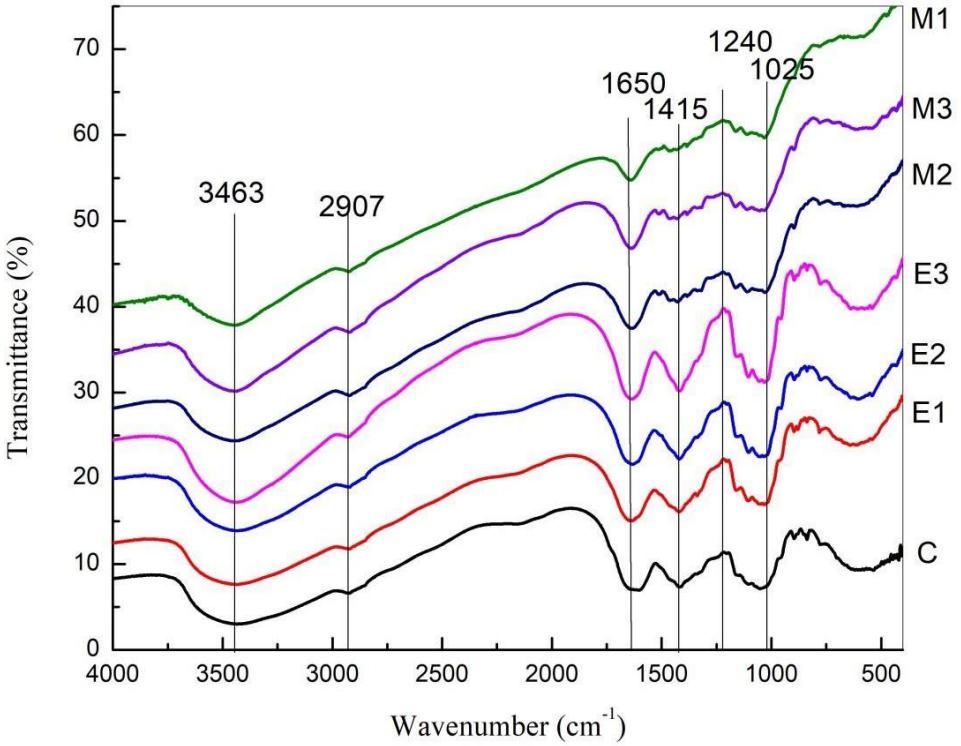

Fig 3.2 Fourier-transformed infrared spectroscopy of unmodified (C) and modified insoluble dietary fiber of pea peel (*Pisum sativum* L.*)* by extrusion (E1, E2, E3) and enzymes (M1, M2, M3) treatment

3.2.3. Crystalline structure

The peak in the XRD graph was typically cellulose crystallinity, with crystalline lines and an unbroken crystal surface [17]. The peaks observed for unmodified samples at 15^0 and 22.5^0 suggest the crystalline cellulose region (Fig 3.3). The prominent intensity peaks were observed in between 20^0 to 25^0 in the modified samples which were due to the presence of cellulose type I and thus having a double helix structure. However, the peaks at 15^0 in unmodified samples were very weak compared to enzyme-modified samples i.e, M1, M2, and M3. The presence of hydrogen bonds and other Van der Waals interactions between neighboring molecules, cellulose showed a crystalline structure. Peaks at 15^0 and 22^0 were stronger in the enzyme treatments of IDF as compared to the extrusion method and the plausible reason might be attributed to the crystalline cellulose area, although their amount decreased after extrusion treatment. In addition, new smaller peaks were also observed in extrusion modified samples (E1, E2, and E3) and that were probably due to the breakdown of the structure and to the high shear and temperature,

and as a result the intensity of peaks decreased [6, 21]. Crystallinity fell dramatically after 25 min, which means that some of the IDF regions were shattered as a result of the alteration.

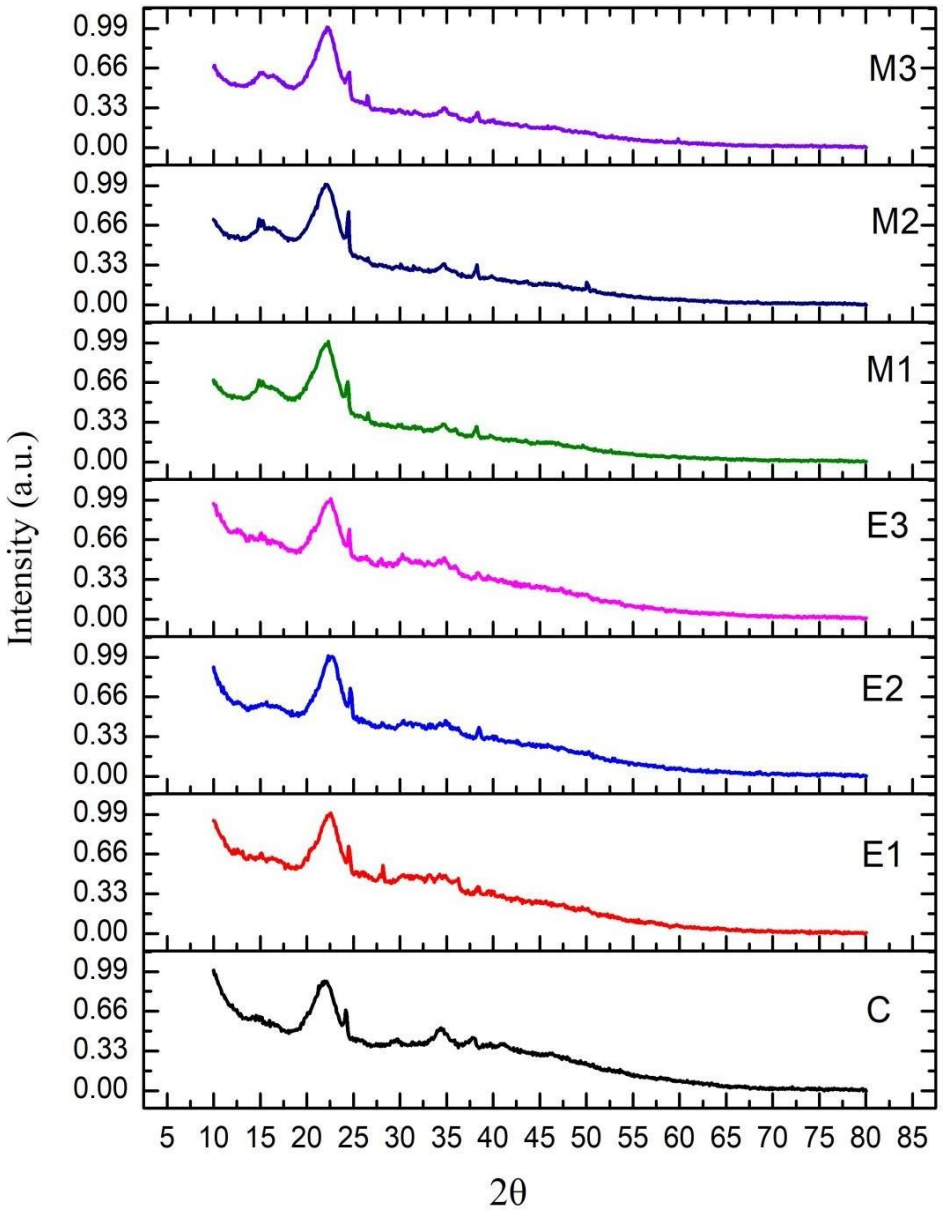

Fig 3.3 X-ray diffraction patterns of unmodified (C) and modified insoluble dietary fiber of pea peel (*Pisum sativum L.)* by extrusion (E1, E2, E3) and enzymes (M1, M2, M3) treatment

.

3.2.4. Surface area analysis

BET technique was used to measure the surface area based on the adsorption of a gas on the solid surface [4] and in the unmodified IDF it was 2.11 m^2/g and is lower than the modified samples. In extrusion modified samples (E1 to E3), the surface area got decreased from 2.895 m^2/g-2.276 m^2/g. The plausible reason might be due to increasing in the extrusion cooking temperature and thereby increased in shear, which reduced the surface area (Table 3.1). However, in enzyme modifications, the value of surface area varied from 11.892 m^2/g -15.358 m^2/g for M1 to M3 that might be due to the change in the concentration ratio of enzymes used. The surface porosity of IDF increased appreciably (Fig 3.1), due to enzyme modification, thus there was an increase in the gas adsorption capacity and resulted significant increase in BET surface area [4].

Table 3.1 Brunauer–Emmett–Teller surface area of pea peel (*Pisum sativum* L.) insoluble dietary fiber (IDF) by extrusion treatment (E1, E2, E3) and enzymes (M1, M2, M3)

Sample type	BET surface area (m^2/ g)	Pore Volume (cm^3/g)	Pore Radius (nm)
C	2.110	0.006	18.001
E1	2.895	0.008	12.388
E2	2.654	0.007	25.734
E3	2.276	0.004	17.131
M1	15.358	0.023	16.135
M2	12.852	0.005	18.056
M3	11.892	0.019	17.985

3.2.5. Thermal properties

The thermal properties of the modified and unmodified IDF were determined by using differential scanning calorimetry (DSC). The unmodified IDF sample had a peak temperature (T_p), an onset temperature (T_o) and an end temperature (T_e) of 118.4^{0}C, 101^{0}C, and 149^{0}C (T_c), respectively. However, after the modification, the peak, onset, and conclusion temperature of IDF decreased noticeably (Table 3.2). During the extrusion and enzyme modifications, structural degradation occurred, and lowered the energy required to break down the intramolecular and intermolecular connections. Apparently, it takes a longer time for small particles to melt the bulk of the sample [25].

Enzyme-modified samples showed lower thermal properties than the unmodified and extrusion treated samples. Enzyme modified samples M3 showed the least thermal stability with 92.3^{0}C and 123.2^{0}C as peak temperature and conclusion temperature, respectively. The IDF structure became porous as a result of the enzyme treatment, which reduces heat resistance and lowers the peak, onset, and conclusion temperatures. The thermogram showed an exothermic peak for all the samples, which might be due to the pyrolysis of polysaccharides (cellulose, hemicellulose, and lignin) and starting with the glycosidic bond breakdown [17, 28].

Table 3.2 Thermal properties of insoluble dietary fiber of pea peel (*Pisum sativum* L.) by extrusion treatment (E1, E2, E3) and enzymes (M1, M2, M3)

Temperature (°C)	C	E1	E2	E3	M1	M2	M3
Peak (Tp)	118.4	114.5	115.9	117.5	95.3	95.9	92.3
Onset (To)	101	78.2	82.5	84.2	55.8	59.6	61.4
Endset (Tc)	149	135.2	138.6	139.0	122.0	127.4	123.2

3.2.6. Thermal decomposition

The thermogravimetric curve mainly represents the degradation or weight loss with respect to temperature as shown in Fig 3.4. When a food product is thermally processed in the industry, thermal stability is a critical factor [19]. Modification showed some noticeable changes in the IDF with respect to unmodified IDF and revealed two-stage degradation behaviour. All the samples showed a weight loss peak (~3%) at around 100^{0}C and could be attributed to the evaporated water. The enzyme-modified sample (M3) had the highest mass loss of 52.84% at 430^{0}C, while the unmodified sample had the lowest mass loss of 39.87% at 375^{0}C. The significant weight loss peak at 250^{0}C occurred because of the degradation of cellulose and hemicellulose [24]. For extrusion modification (E1-E3), the first significant weight loss occurred between 350 to 380^{0}C, and respective weight loss were 40.46%, 40.01%, and 41.13%. The increase in the degradation temperature could be attributed to the more compact and stronger IDF structure. At 430^{0}C, enzyme-modified samples (M1-M3) showed mass loss by 49.09%, 52.76%, and 52.84%, respectively. It is possible that cellulase and xylanase hydrolysis led to the IDF particles loosening on the surface and enhancing the porosity, and thus

increasing the mass transfer. Among all the modified samples, M3 was found to be the highest in thermal stability and the probable reason could be due to the intermolecular cross-links developed by enzyme treatment which increased the surface charges and colloidal dispersibility [28, 17]. There was a relatively low reduction between 400^0C to 600^0C indicated that structure of the IDF network did not change with temperature.

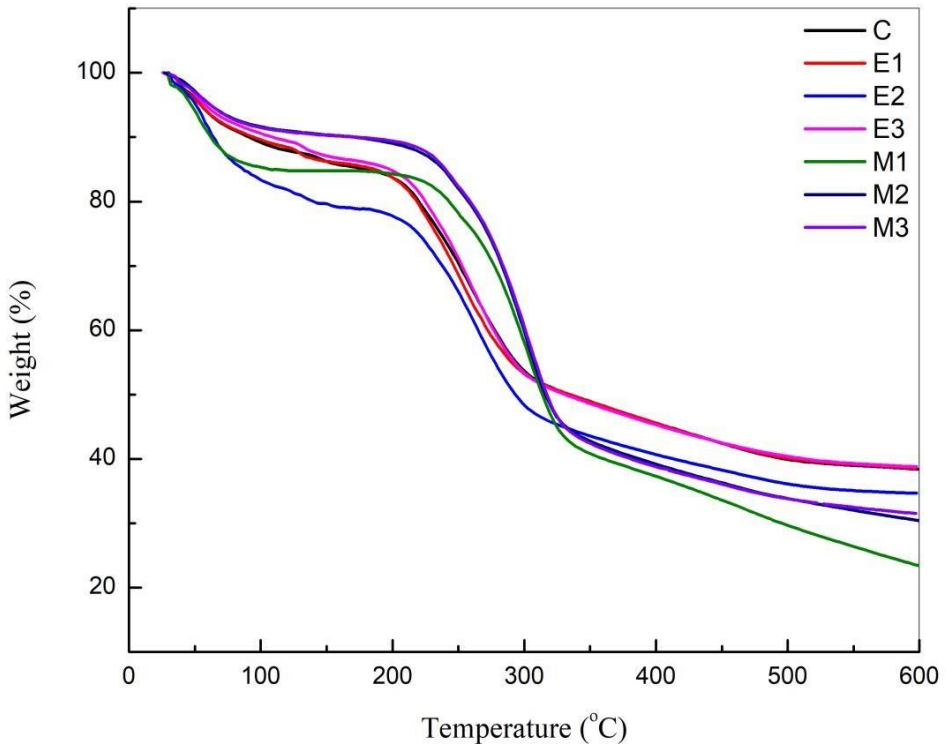

Fig 3.4 Thermal decomposition behaviour of unmodified (C) and modified dietary fiber of pea peel (*Pisum sativum* L.) by extrusion (E1, E2, E3) and enzymes (M1, M2, M3) treatment

3.2.7. Physicochemical and functional properties

The physicochemical properties of pea peel modified and unmodified IDF are presented in Table 3.3. The WRC of dietary fiber is an important indicator to evaluate the hydrolytic properties in the gastrointestinal system which can increase the faecal volume by promoting fermentation in the gut and can prolong the satiety. The WRC of unmodified sample was 0.12 g/g and extrudate sample varied from 0.32-1.52 g/g. However, a significant increase was observed for the enzyme-modified sample from 2.83

to 3.25 g/g. The IDF destruction caused by the thermal and mechanical processing by extrusion enhanced the binding site and thereby increasing the WRC. Consequently, due to enzyme hydrolysis, the spaces or cavities shown on the surface of the dietary fiber developed significantly (Fig 3.1) and thus increasing the WRC.

There was a significant ($p<0.05$) increase in swelling capacity (SC) of the modified IDF as compared to the unmodified sample (Table 3.3). The SC value of the unmodified sample C was 4.53 mL/g, whereas for E1-E3 and M1-M3 results varied from 4.82-5.76 mL/g and 7.83-8.89 mL/g, respectively. The more β-glycosidic bond lysed by the enzyme, the more will be the hydration area due to the exposed hydrogen bonds and thus could increase the SC and able to provide more satiety [9, 30].

The oil adsorption capacity (OAC) is a parameter responsible to assess the capacity of dietary fiber to reduce serum cholesterol and inhibit the accumulation of fat. The OAC for enzyme treatment increased significantly ($p<0.05$) but a smaller change was reported for extrusion modified sample (Table 3.3). The OAC varied from 2.70-2.83 g/g and 3.01-3.25 g/g for E1-E3 and M1-M3 samples, respectively. A similar kind of observation was also reported by Song et al. [24] for citrus fiber.

The cation exchange capacity (CEC) of the unmodified sample was 0.20 g/g and after modification a significant ($p<0.05$) increase in the CEC was noticed (Table 3.3). Due to the mechanical action by the extrusion treatment, some of the side chains might expose which improved the CEC. Enzyme treated samples showed significantly high CEC in contrast to the unmodified and extrusion treated samples. The porosity of the IDF network increased in the presence of cellulase and xylanase and resulting in a considerable rise in the CEC and is reported to reduce the Na^+/Ka^+ ratio and thus helping to lower blood pressure [32].

The glucose adsorption capacity (GAC) of the IDF samples varied significantly ($p<0.05$) after the modification (Table 3.3). The GAC of enzyme modified fiber significantly increased compared to the extrusion modified fiber and that could be due to porosity or cavities present on the surface that helped to entrap the glucose molecules provided by modification (Fig 3.1) [3, 14].

Table 3.3 Effects of modification of insoluble dietary fiber (IDF) of pea peel (*Pisum sativum* L.) by extrusion treatment (E1, E2, E3) and enzymes (M1, M2, M3) on the physical and functional properties

Samples	WRC (g/g)	SC (mL/g)	OAC (g/g)	CEC (mmol/g)	AC (mmol/L)
C	0.12 ± 0.01^{a}	4.53 ± 0.29^{a}	2.71 ± 0.01^{a}	0.20 ± 0.02^{a}	1.90 ± 0.21^{a}
E1	1.52 ± 0.08^{c}	5.76 ± 0.32^{b}	2.83 ± 0.07^{b}	0.32 ± 0.05^{b}	2.56 ± 0.38^{c}
E2	1.51 ± 0.09^{c}	5.75 ± 0.29^{b}	2.81 ± 0.06^{b}	0.35 ± 0.06^{c}	2.68 ± 0.17^{d}
E3	0.32 ± 0.02^{b}	4.82 ± 0.23^{a}	2.70 ± 0.07^{a}	0.40 ± 0.05^{e}	2.10 ± 0.22^{b}
M1	3.25 ± 0.17^{f}	8.89 ± 0.16^{e}	3.25 ± 0.01^{e}	0.86 ± 0.08^{g}	4.73 ± 0.52^{g}
M2	3.01 ± 0.20^{e}	8.35 ± 0.27^{d}	3.21 ± 0.02^{d}	0.79 ± 0.09^{f}	4.42 ± 0.55^{f}
M3	2.83 ± 0.12^{d}	7.83 ± 0.38^{c}	3.01 ± 0.04^{c}	0.62 ± 0.06^{d}	4.23 ± 0.58^{e}

C: Pea peel unmodified insoluble dietary fiber; E1: Modified IDF by extrusion treatment at 130°C, E2: Modified IDF by extrusion treatment at 140°C, E3: Modified IDF by extrusion treatment at 170°C; M1: Modified IDF by Cellulase:Xylanase, 90:21U/g, M2: Modified IDF by Cellulase:Xylanase, 65:65 U/g, M3: Modified IDF by Cellulase:Xylanase, 21:90 U/g.

WRC, Water retention capacity; SC, Swelling capacity; OAC, Oil retention capacity; CEC, Cation exchange capacity.

All data are averages of three measurements with standard deviation with significant difference ($p < 0.05$).

3.2.8. Glucose dialysis retardation index

The glucose dialysis retardation index (GDRI) is an *in vitro* index that could be used to anticipate how a fiber would affect the delay in glucose absorption in the gastrointestinal system. Unmodified and modified samples could bind to glucose in 100 mmol/L glucose concentration (Fig 3.5). The GDRI of all the samples gradually increased in the first 30 min and then decreased, indicating a strong property to control postprandial blood glucose levels. GDRI decreased as the extrusion temperature increased, which is in agreement with GAC. The enzyme-modified samples, on the other hand, had a considerably higher GDRI ($p<0.05$). It has been proposed that increase in porosity and specific surface area of dietary fiber could increase the entrapment of glucose molecules inside the fiber network and hence boosting the GDRI [28].

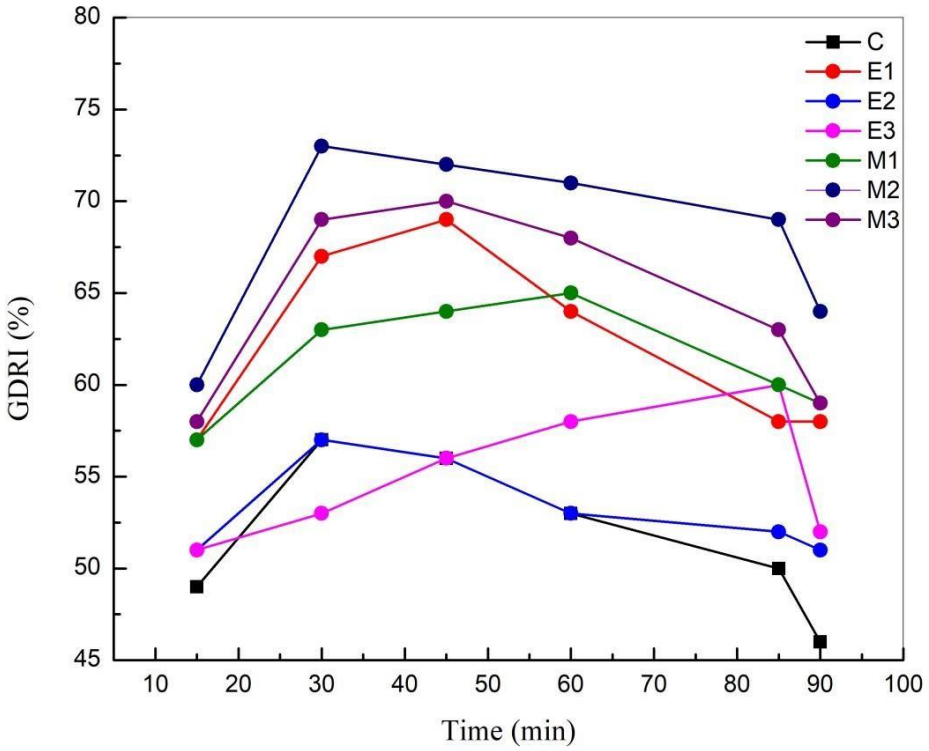

Fig 3.5 Glucose dialysis retardation index (GDRI) curve of insoluble dietary fiber (IDF) of pea peel (*Pisum sativum* L.) (i) control (a) 'C', (ii) modified IDF by extrusion treatment: (b) E1, (c) E2, (d) E3 and (iii) modified IDF by enzymes treatments: (e) M1, (f) M2, (g) M3

3.2.9. Color of yogurt with modified and unmodified IDF

The inclusion of modified IDF to yogurt completely varied in terms of colour with the yogurt containing unmodified dietary fiber supplementation (Table 3.4). The control sample showed L* value of 85.52 which was less than the modified rich yogurt samples (i.e., YE1-YE3 and YM1-YM3). It can be attributed to the incorporation of unmodified IDF to control sample. Among the modified yogurt types, YE1-YE3 showed more dark color as compared to the YM1-YM3 fortified yogurt samples. This could be due to the change in surface color in presence of high temperature during the extrusion (i.e., YE1-YE3). The a* value for control (YC), YM1-YM3 and YE1-YE3 samples varied from 0.52±0.03, -0.44 to -0.74 and 0.22 to 0.62, respectively. The b value for all modified samples varied from 8.03 to 11.01 and it was 17.39 in the unmodified (control). Color intensity (C*) increased from 7.55 to 17.39 with an increase in color perception (h°). However, yogurt with enzyme-modified IDF showed no significant change in L*, C*, or (h°) values, which might be due to the enzyme-modified fiber's light colour that blends

well with yoghurt colour. The degree of modification, varying temperatures, and enzyme treatments used for the IDF might explain the colour differences amongst the samples.

Table 3.4 Color value of yogurt fortified with modified and unmodified insoluble dietary fiber (IDF) of pea peel (*Pisum sativum* L.)

Yogurt types	L*	a*	b*	Chroma (C*)	Hue angle (h°)
YC	85.52±0.1^{a}	0.52±0.03^{b}	17.39±0.91^{f}	17.39±0.90^{f}	88.28±0.9^{e}
YE1	86.51±0.9^{b}	0.22±0.05^{a}	11.01±0.71^{f}	11.01±0.51^{f}	88.85±0.9^{e}
YE2	84.57±1.2^{a}	0.62±0.09^{d}	10.11±0.40^{e}	10.12±0.48^{e}	86.49±0.4^{c}
YE3	87.06±0.7^{c}	0.73±0.08^{e}	9.96±0.41^{d}	9.98±0.46^{d}	85.80±1.1^{b}
YM1	88.92±0.6^{d}	-0.44±0.05^{b}	8.79±0.39^{c}	8.80±0.38^{c}	-87.13±1.0^{d}
YM2	90.42±0.7^{e}	-0.56±0.06^{c}	8.03±0.41^{b}	8.04±0.31^{b}	-86.01±0.9^{c}
YM3	88.59±0.9^{d}	-0.74±0.03^{e}	8.11±0.42^{b}	8.14±0.32^{b}	-84.78±1.1^{a}

YC: Yogurt with unmodified IDF; YE1: Yogurt with E1 IDF, YE2: Yogurt with E2 IDF, YE3: Yogurt with E3 IDF; YM1: Yogurt with M1 IDF, YM2: Yogurt with M2 IDF, YM3: Yogurt with M3 IDF.
All data are averages of three measurements with standard deviation with significant difference ($p < 0.05$).

3.2.10. Rheological properties of yogurt with modified IDF

Typical curves of storage modulus (G′) of yogurt incorporated with modified and unmodified IDF in control sample as a function of frequency (ω) has been observed (Fig 3.6). The dynamic G′ is a measure of the energy stored in the material and recovered from it per cycle of sinusoidal deformation while the loss modulus (G″) is a measure of the energy dissipated or lost per cycle. The addition of unmodified and modified IDF changed the oscillatory rheological behaviour of yoghurt dramatically. The viscoelastic behaviour of the G′ of the modified IDF yogurt was linear (Fig 3.6). Yogurt had a lower frequency dependence of moduli than fluid, indicating a more rigid system. This might be attributed to the yogurt had an IDF, which enhanced supramolecular interaction during gel formation. FT-IR studies revealed that the modified fiber increased the storage modulus of the yogurt, resulting in a larger binding site. Incorporation of E1 to E3 resulted in a decrease in G′, suggesting a loss of gel strength, as well as a complex behaviour in the presence of M1, M2, and M3.

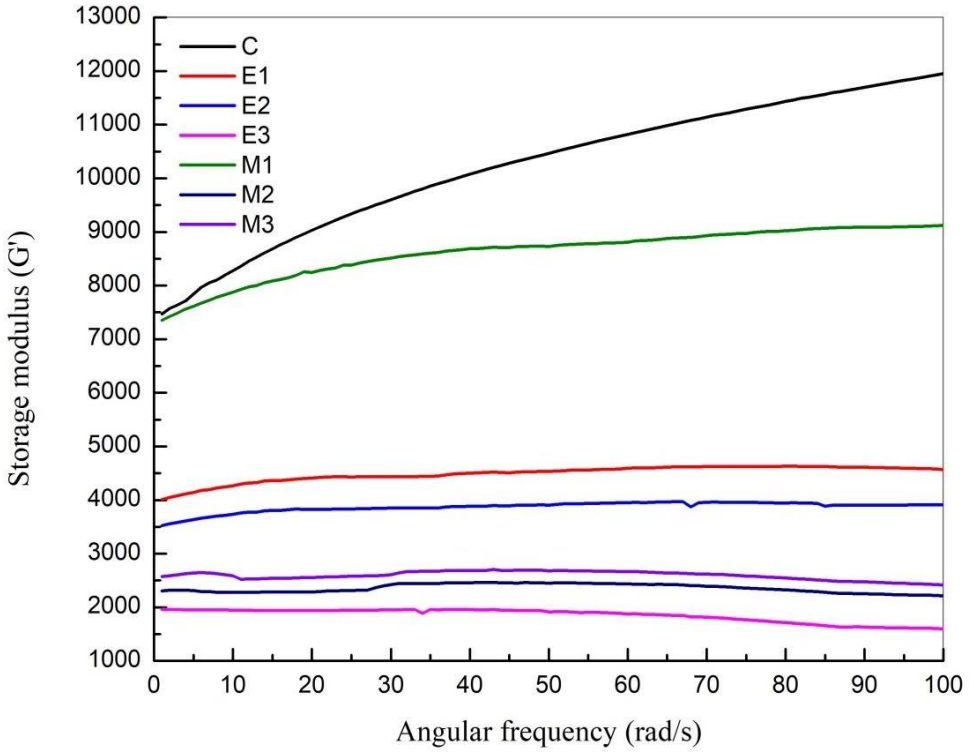

Fig 3.6 Rheological curve of yogurt combined with modified and unmodified insoluble dietary fiber (IDF) of pea peel (*Pisum sativum* L.) [(i) Yogurt with unmodified IDF; YC (ii) yogurt with modified IDF by extrusion treatment; YE1, YE2, YE3 and (iii) yogurt with modified IDF by enzymes treatments; YM1, YM2, YM3]

Table 3.5 Dietary fiber content before and after modification treatment for pea peel

Treatment	Green pea peel dietary fiber		
	IDF (%)	SDF (%)	TDF (%)
C	63.92±2.64 ^{c}	14.00±0.87^{a}	77.92±3.51^{a}
E1	62.67±2.31^{b}	15.07±0.92 ^{b}	77.74±3.43^{a}
E2	61.42±2.09^{a}	16.45±0.91 ^{b}	77.87±3.42^{a}
E3	62.98±2.41^{b}	14.96±0.93^{a}	77.94±3.19^{a}
M1	62.76±2.12^{b}	24.45±1.08^{c}	87.21±4.04^{b}
M2	61.98±2.04^{a}	28.54±1.09^{e}	90.52±5.01^{c}
M3	62.34±2.32 ^{b}	26.04±1.10^{d}	88.38±4.21^{b}

C: Pea peel unmodified insoluble dietary fiber; E1: Modified IDF by extrusion treatment at 130°C, E2: Modified IDF by extrusion treatment at 140°C, E3: Modified IDF by extrusion treatment at 170°C; M1: Modified IDF by Cellulase:Xylanase, 90:21U/g, M2: Modified IDF by Cellulase:Xylanase, 65:65 U/g, M3: Modified IDF by Cellulase:Xylanase, 21:90 U/g.

All data are averages of three measurements with standard deviation with significant difference ($p < 0.05$).

3.3. Conclusion

Extrusion and enzyme treatments were reasonably effective methods for modifying the IDF in pea peels. Extrusion with various process conditions and a combination of cellulase and xylanase significantly improved the physicochemical and functional properties by destroying the crystalline region and enzymes tend to attack the amorphous portion. The IDF's microstructure was destroyed more rigorously which created more oligosaccharides due to specificity of enzymes. The crystallinity improved due to the disruption in the molecular structure. In comparison to the unmodified IDF, the BET surface area was much higher and WRC, SC, OAC, and CEC of the modified IDF improved significantly ($p < 0.05$). The oscillatory rheological behaviour of the yogurt changed significantly when the modified IDF was added. Extrusion and enzyme treatments might be used to customise the IDF and can be used it as a functional food element in dairy and other foods.

References

[1] Aktas-Akyildiz, E., Masatcioglu, M. T., & Köksel, H. Effect of extrusion treatment on enzymatic hydrolysis of wheat bran. *Journal of Cereal Science*, *93*, 102941, 2020.

[2] Bader Ul Ain, H., Saeed, F., Khan, M. A., Niaz, B., Khan, S. G., Anjum, F. M., and Hussain, S. Comparative study of chemical treatments in combination with extrusion for the partial conversion of wheat and sorghum insoluble fiber into soluble. *Food Science & Nutrition*, *7*(6), 2059-2067, 2019.

[3] Chau, C. F., Wang, Y. T., & Wen, Y. L. Different micronization methods significantly improve the functionality of carrot insoluble fiber. *Food Chemistry*, *100*(4), 1402-1408, 2007.

[4] Chen, L., Batchelor-McAuley, C., Rasche, B., Johnston, C., Hindle, N., and Compton, R. G. Surface area measurements of graphene and graphene oxide samples: Dopamine adsorption as a complement or alternative to methylene blue? *Applied Materials Today*, *18*, 100506, 2020.

[5] Cai, W. D., Zhu, J., Wu, L. X., Qiao, Z. R., Li, L., and Yan, J. K. Preparation, characterization, rheological and antioxidant properties of ferulic acid-grafted curdlan conjugates. *Food Chemistry*, *300*, 125221, 2019.

[6] Fang, D., Wang, Q., Chen, C., Li, Z., Li, S., Chen, W., and Zheng, Y. Structural characteristics, physicochemical properties and prebiotic potential of modified dietary fiber from the basal part of bamboo shoot. *International Journal of Food Science & Technology*, *56*(2), 618-628, 2021.

[7] Guo, F., Xiong, H., Wang, X., Jiang, L., Yu, N., Hu, Z., ... & Tsao, R. Phenolics of green pea (Pisum sativum L.) hulls, their plasma and urinary metabolites, bioavailability, and in vivo antioxidant activities in a rat model. *Journal of Agricultural and Food Chemistry*, *67*(43), 11955-11968, 2019.

[8] Hanan, E., Rudra, S. G., Sagar, V. R., & Sharma, V. Utilization of pea pod powder for formulation of instant pea soup powder. *Journal of Food Processing and Preservation*, *44*(11), e14888, 2020.

[9] Huang, Y. L., & Ma, Y. S. The effect of extrusion processing on the physiochemical properties of extruded orange pomace. *Food Chemistry*, *192*, 363-369, 2016.

[10] Jia, M., Chen, J., Liu, X., Xie, M., Nie, S., Chen, Y., and Yu, Q. Structural characteristics and functional properties of soluble dietary fiber from defatted rice bran obtained through *Trichoderma viride* fermentation. *Food Hydrocolloids*, *94*, 468-474, 2019.

[11] Kumari, T., & Deka, S. C. Potential health benefits of garden pea seeds and pods: A review. *Legume Science*, *3*(2), e82, 2021

[12] Kumari, T., Das, A. B., & Deka, S. C. Impact of extraction methods on functional properties and extraction kinetic of insoluble dietary fiber from green pea peels: A comparative analysis. *Journal of Food Processing and Preservation*, e16476, 2022.

[13] Lin, D., Long, X., Huang, Y., Yang, Y., Wu, Z., Chen, H., and Tu, Z. Effects of microbial fermentation and microwave treatment on the composition, structural characteristics, and functional properties of modified okara dietary fiber. *LWT*, *123*, 109059, 2020.

[14] Liu, Y., Zhang, H., Yi, C., Quan, K., & Lin, B. Chemical composition, structure, physicochemical and functional properties of rice bran dietary fiber modified by cellulase treatment. *Food Chemistry*, *342*, 128352, 2021.

[15] Liu, H., Gong, F., Wei, F., Lai, F., Zhang, X., & Wu, H. Artificial simulated gastrointestinal digestion of four carbohydrates containing beta-d-1→ 4 linkages and new GC-TQ/MS-MS method for characterising released monosaccharides. *International Journal of Food Science & Technology*, *53*(8), 1992-2005, 2018.

[16] Ma, S., Ren, B., Diao, Z., Chen, Y., Qiao, Q., & Liu, X. Physicochemical properties and intestinal protective effect of ultra-micro ground insoluble dietary fiber from carrot pomace. *Food & Function*, *7*(9), 3902-3909, 2016.

[17] Ma, Q., Ma, Z., Wang, W., Mu, J., Liu, Y., Wang, J., and Sun, J. The effects of enzymatic modification on the functional ingredient-dietary fiber extracted from potato residue. *LWT*, *153*, 112511, 2022.

[18] Maphosa, Y., & Jideani, V. A. (2016). Dietary fiber extraction for human nutrition—A review. *Food Reviews International*, *32*(1), 98-115, 2016.

[19] Nawrocka, A., Szymańska-Chargot, M., Miś, A., Wilczewska, A. Z., & Markiewicz, K. H. Effect of dietary fiber polysaccharides on structure and thermal properties of gluten proteins–A study on gluten dough with application of FT-Raman spectroscopy, TGA and DSC. *Food Hydrocolloids*, *69*, 410-421, 2017.

[20] Qiao, H., Shao, H., Zheng, X., Liu, J., Liu, J., Huang, J., and Guan, W. Modification of sweet potato (*Ipomoea batatas* Lam.) residues soluble dietary fiber following twin-screw extrusion. *Food Chemistry*, *335*, 127522, 2021

[21] Ren, F., Feng, Y., Zhang, H., & Wang, J. Effects of modification methods on microstructural and physicochemical characteristics of defatted rice bran dietary fiber. *LWT*, *151*, 112161, 2021.

[22] Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. Physicochemical, textural and rheological properties of probiotic yogurt fortified with fiber-rich pineapple peel powder during refrigerated storage. *LWT*, *65*, 978-986, 2016.

[23] Schmid, V., Trabert, A., Schäfer, J., Bunzel, M., Karbstein, H. P., & Emin, M. A. Modification of apple pomace by extrusion processing: Studies on the composition, polymer structures, and functional properties. *Foods*, *9*(10), 1385, 2020.

[24] Song, L. W., Qi, J. R., Liao, J. S., & Yang, X. Q. Enzymatic and enzyme-physical modification of citrus fiber by xylanase and planetary ball milling treatment. *Food Hydrocolloids*, *121*, 107015, 2021.

[25] Van Dooren, A. A., & Müller, B. W. Effects of heating rate and particle size on temperatures and specific enthalpies in quantitative differential scanning calorimetry. *Thermochimica Acta*, *54*(1-2), 115-129, 1982.

[26] Wang, H. O., Liu, S. H., Zhou, X. J., Yang, X. Y., Gao, Q., Tanokura, M., & Xue, Y. L. Treatment with hydrogen peroxide improves the physicochemical properties of dietary fibers from Chinese yam peel. *International Journal of Food Science & Technology*, *55*(3), 1289-1297, 2020.

[27] Wang, X., Zhang, Y., Li, Y., Yu, H., Wang, Y., & Piao, C. Insoluble dietary fiber from okara (soybean residue) modified by yeast Kluyveromyces marxianus. *LWT*, *134*, 110252, 2020.

[28] Yang, T., Yan, H. L., & Tang, C. H. Wet media planetary ball milling remarkably improves functional and cholesterol-binding properties of okara. *Food Hydrocolloids*, *111*, 106386, 2021

[29] Yang, B., Wu, Q., Song, X., Yang, Q., & Kan, J. Physicochemical properties and bioactive function of Japanese grape (*Hovenia dulcis*) pomace insoluble dietary fiber modified by ball milling and complex enzyme treatment. *International Journal of Food Science & Technology*, *54*(7), 2363-2373, 2019.

[30] Yu, G., Bei, J., Zhao, J., Li, Q., & Cheng, C. Modification of carrot (*Daucus carota* Linn. var. Sativa Hoffm.) pomace insoluble dietary fiber with complex enzyme method, ultrafine comminution, and high hydrostatic pressure. *Food Chemistry*, *257*, 333-340, 2018.

[31] Zhong, L., Fang, Z., Wahlqvist, M. L., Hodgson, J. M., & Johnson, S. K. Extrusion cooking increases soluble dietary fiber of lupin seed coat. *LWT*, *99*, 547-554.

[32] Zhou, D., Liu, J., Liu, S., Liu, X., Tang, X., & Lv, X. Characterisation of alkaline and enzymatic modified insoluble dietary fiber from *Undaria pinnatifida*. *International Journal of Food Science & Technology*, *55*(12), 3533-3541, 2020.