CHAPTER-2

Impact of extraction methods on functional properties and extraction kinetic of insoluble dietary fiber from green pea peels: A comparative analysis

2.1. Introduction

Pea (*Pisum sativum* L.) is one of the most important legumes in the world and rich in nutritious compounds such as carbohydrates, proteins and dietary fibers and other phytochemical compounds and has been important in diet [18, 26]. However, during processing a huge amount of waste is generated in terms of peel. Green pea peels (GPP) are the under-exploited solid waste by-product after removal of peas and have immense health benefits. GPP is an outstanding source of dietary fiber, vitamins and minerals [18]. About 35% to 40% solid waste of GPP is generated during pea processing that represents a key issue in terms of environmental problems [24]. Proximate analysis of GPP waste contains 26% cellulose, 20.5% hemicellulose, 3.92% lignin, 20.2% crude protein, and 5% ash [18]. There is very little insightful research conducted to the dietary fiber of GPP.

Dietary fiber (DF) is a vital chemical component needed by human that can be explicated as the mixture of non-starch polysaccharides, enriched in whole grains, nuts, fruits and vegetables. During the enzymatic digestion it shows the resistance capacity in the small intestine [23]. Based on water solubility, the total dietary fiber (TDF) can be sub-categorised into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) [35, 16, 14]. Many health significant diseases, including cardiovascular disease, type-2 diabetes, obesity as well as constipation and gastrointestinal disorders have been reduced with the intake of dietary fiber [29]. The beneficial effects of fiber have attracted the attention of researchers and the industries to develop a potential market for dietary fiber enriched functional food products. Therefore, there is a huge demand to extract the dietary fiber from the waste plant materials.

There are various types of extraction methods viz., wet processing methods (e.g., conventional wet milling, alkali wet milling, enzymatic wet milling, modified wet milling), physical and microbial methods, gravimetric methods (e.g., nonenzymatic-gravimetric method, enzymatic-gravimetric method, enzyme assisted chemical method)

[24, 7]. The various extraction processing methods significantly affect the yield, structure, physicochemical properties and composition of dietary fiber and which in turn directly influence its functional properties. Alkali extraction process is highly followed by the various industries as it removes the proteins from the residue by providing high purity dietary fiber [30]. In order to obtain maximum yield with high purity, recent processing conditions are shifted towards the application of ultrasound in extraction processes. Starch and protein fractions are separated by the use of ultrasound extraction method to increase the extraction yield and also to reduce the extraction time [37]. However, there is no insightful study on the extraction of IDF from GPP by various extraction methods on the functional as well as structural properties.

DF has been shown to reduce many chronic diseases by limiting blood sugar level, lowering fat, preventing cancer and many more. The gut microbial communities are also highly affected by the dietary fiber intake [22, 36]. DF constitutes about 60-80% IDF in most of the plant-based products, which is mainly accountable for stimulating bowels function [14,15]. IDF showed various beneficial effects such as intestinal peristalsis promotion, increase in faecal volume and elimination of toxic elements and other heavy metals whereas SDF showed biological, physiological, antioxidant properties. DFs mainly include IDF, which could be utilised in an effective nutritional way [17]. Due to the complex nature of IDF it has limited use but to minimise the wastage of IDF many industries have used the extraction method followed by modification, to provide economic feasibility and incorporating them in other food products, which can promote health benefit and wellness among consumers. Incorporation of high amount of IDF in food model has increased product firmness during storage [19]. IDF in the food lowers transit time, as well as restrict the contact time of harmful substances with the intestinal walls [18,19]. Various report suggested that application of IDF in food processing industries use numerous emerging technologies to modify and enhance its physiochemical properties [35, 6].

Therefore, the present study was primarily focused to identify the suitable extraction method for the pea peel IDF as well as its effects of extraction parameters on the physical, functional and morphological properties. The IDF was optimised in ultrasound assisted alkaline extraction method and further the process was compared with conventional process and the properties of IDF were assessed on the basis of

physicochemical, thermal, functional and structural properties.

2.2. Materials and methods

2.2.1. Raw materials

Green peas (*Pisum sativum* L.) were purchased from Tezpur local market, Assam, India and immediately the green pea peels (GPP) were separated and washed. The peels were then dried in tray dryer (BDI-51, Labotech, Delhi, India) at 55°C for 12 h and sieved through the 80 mesh (180 micron) followed by grinding and stored at room temperature (25±2°C) for further analysis.

2.2.2. Sample preparation

The dried GPP was deoiled using 95% ethanol as well as to remove organic acids, low molecular weight sugars, or inorganic salts [28]. The GPP was mixed with ethanol (1:4 w/v) and stirred at 150 rpm for 20 min. The mixture was centrifuged (Eppendorf Centrifuge 5430R, Germany) at 4000 rpm (418.8 rad/s) for 20 min. Thereafter, the deoiled GPP residue was dried at 50°C for 3 h in tray drier (Labotech, BDI-51, India).

2.2.3. Alkaline extraction

The alkaline extraction of insoluble dietary fiber (ADF) was carried out according to the method of Zhang et al. [37]. Deoiled GPP (2.0 g, dry basis) was suspended in 0.5 M NaOH (1:25, solid/liquid) and stirred at 500 rpm using laboratory stirrer (RQT-127D, Remi, Maharashtra, India) for 30 min at 50°C. The mixture was neutralized using 0.5 M HCl followed by centrifugation at 4000 g for 30 min. The supernatant was separated, and residue was dried at 40°C for 12 h to obtain the alkaline extracted IDF.

2.2.4. Ultrasound assisted extraction

The IDF was extracted using ultrasound assisted extraction (UDF) method. Deoiled GPP was suspended in distilled water (1:25, solid/liquid), stirred for 30 min at 50°C. It was further sonicated at 30 kHz for 30 min at a temperature range of 40-50°C using probe type ultrasonicator (Q700-220 Digital Sonicator, Qsonica LLC, USA).

2.2.5. Ultrasound assisted alkaline extraction

For ultrasound assisted alkaline extraction (ODF), the GPP (2.0 g, dry basis) was

weighed and transferred to an extraction tube. For the extraction process, NaOH concentration (0.6% to 1.4%), extraction time (15 to 35 min), solid to liquid ratio (1:10 to 1:50), and ultrasonic amplitude (20 to 60%) were used as independent parameters. The experiment was designed according to rotatable central composite design. Altogether 30 experiments were conducted for the process optimization. For extraction, NaOH solution was added to the extraction tube, and each extraction was performed under controlled ultrasound-assisted alkaline conditions using probe type ultrasonicator (Q700-220 Digital Sonicator, Qsonica LLC, USA). Immediately after extraction, the suspension was centrifuged at 4000 g for 30 min (Eppendorf Centrifuge 5430R, Germany). Residue was collected as IDF followed by ethanol wash (single) for separation of soluble dietary fiber (SDF) fraction. Centrifugation was done at 8000 g for 30 min (Eppendorf Centrifuge 5430R, Germany) and the precipitate was collected and then freeze dried (Lyolab Freeze Lab, Lyophilization Systems Inc., USA).

2.2.6. Water holding capacity

The water holding capacity (WHC) of the IDF was determined using 1 g of sample (M_1) and was taken in a graduated centrifuge tube (M_0) followed by addition of 30 mL of water and stirred for 18 h and the mixture was centrifuged at 3000 g for 20 min. The residue was weighed and oven-dried at 105°C to constant weight (M_2) [5]. WHC was calculated according to the following eq. (1):

$$WHC\ (g/g) = \frac{M_2 - M_0}{M_1} \times 100\% \quad (1)$$

where, M_0 - weight of centrifuge tube (g), M_1 - dry sample weight (g), M_2 - centrifuge tube with wet sample weight (g).

2.2.7. Oil holding capacity

The oil holding capacity (OHC) of the sample was estimated and for this 0.5 g of sample (M_1) was accurately weighed into a centrifuge tube (M_0) and mixed with 5 mL of olive oil. The sample was kept at 4°C for 1 h and then centrifuged at 4000 g for 15 min. After the supernatant was decanted, the residue was collected and weighed (M_2) [38]. The OHC was calculated as the ratio of the quantity of oil to the initial dry weight of the residue as shown in the following eq (2):

$$OHC\ (g/g) = \frac{M_2 - M_0}{M_1} \times 100\% \quad (2)$$

where, M_0 - centrifuge tube weight (g), M_1 - dry weight sample (g), M_2 - centrifuge tube weight with sample wet weight (g).

2.2.8. Swelling capacity

For swelling capacity, 1 g ($_0$) of IDF was taken into a 25 mL measuring cylinder, and the volume of dietary fiber before swelling was recorded (V_0). Following this, 15 mL of distilled water was added to completely immerse the sample. The mixture was soaked for 24 h and recorded the volume after swelling (V_1) [10].

$$SC\ (ml/g) = \frac{V_1 - V_0}{M_0} \times 100\% \quad (3)$$

where, V_0 - volume of sample before water absorption (mL), V_1 - volume of sample after water absorption (mL), and M_0 - sample weight (g).

2.2.9. Colour

The L, a, and b colour values of the extracted IDF were measured using a Hunter Color Measurement Spectrophotometer (UltraScan VIS, Hunter Lab) after standardization.

2.2.10. Scanning electron microscopy

The surface morphology of the extracted IDF was studied using scanning electron microscopy (JSM-6390LV, JEOL, Japan). The analysis was conducted at 20 kV and 1,500 magnifications [20].

2.2.11. Fourier transfer-infrared spectrometry

The functional groups of the extracted IDF samples were determined using spectrometer (Nicolet Instruments 410 FTIR, Thermo Scientific, USA). Spectra were attained with a resolution of 4 cm^{-1} over 400–4,000 cm^{-1} range.

2.2.12. Thermal properties

The thermal properties of the extracted insoluble dietary fiber were determined using differential scanning calorimetry (DSC; Q200 TA Instruments, New Castle, DE, USA). Approximately 5 mg samples were sealed in aluminium pans. The temperature was varied from 20°C to 300°C at 5°C/min. A sealed empty pan was used as a reference. Onset transition temperature (T_o), peak transition temperature (T_p), and final transition temperature (T_c) were recorded.

2.2.13. Thermal decomposition

The thermal decomposition behaviour of the dietary fiber powder was determined using thermogravimetric analysis (TGA), (NETZCH TG 209F1, Libra, Germany) as per the method described by Zhang et al. [37] with certain modification under following condition. Sample (5 mg) was performed in a nitrogen atmosphere where temperature ranged from 25 to 500°C at a rate of 10°C/min.

2.2.14. Extraction kinetics

The extraction kinetics was described by the Peleg's model as follows:

$$C(t) = C_0 + \frac{t}{K_1 + K_2 t} \quad (4)$$

where, $C(t)$ is the concentration of insoluble dietary fiber at time t, t is the extraction time (min), C_0 as the initial concentration of IDF at time $t = 0$, K_1 is Peleg's rate constant and K_2 is Peleg's capacity constant or constant of extraction extent [4]. Since C_0 in all experimental runs was zero; Eq. 5 used in the final form.

$$C(t) = \frac{t}{K_1 + K_2 t} \quad (5)$$

2.2.15. Experimental design

The experiments were designed according to rotatable central composite design (RCCD). There were four independent parameters such as solid to liquid ratio, ultrasonic amplitude, NaOH concentration, and sonication time. The optimization of extraction process was carried out based on two dependent parameters such as total dietary fiber (TDF) and insoluble dietary fiber (IDF). Altogether 30 experiments were conducted

before optimization of the extraction process.

The quadratic polynomial equation obtained from the experimental data to predict the higher IDF in terms of coded variables is presented below.

$$y = A_0 + \sum_{i-1}^{n} A_i x_i + \sum_{i-1}^{n} A_{ii} x_i^2 \sum_{i-1}^{n} \sum_{j-1} A_{ij} x_{ij} + \varphi \quad (6)$$

where, y and x are the dependent and independent variables, respectively. A_0, A_i, A_{ii}, and A_{ij} are the regression coefficients, x_i and x_j represent the linear correlation, x_i^2 and x_{ij} represent the nonlinear correlation and interactive relation between independent variables.

2.2.16. Statistical analysis

2.3. Results and discussion

2.3.1. Response surface analysis

The effect of various independent parameters on the extraction of the TDF and IDF has been illustrated in Fig.2.1. It could be seen from the figures that all the independent parameters evinced significant ($p \leq 0.05$) effect on the extraction of the TDF and IDF from GPP.

Fig. 2.1(a) and (b) represents the relation of the independent variables with IDF. The amount of IDF increased from 52.74 to 81.90% with the increase of amplitude (30-50%). Similar kind of result was also observed for the extraction time. The increase of yield might be attributed to increase in the transfer rate form disruption of cells and structures [37]. Increasing the extraction time allows the solvent to be in contact with the material for a longer period, and there by higher diffusion rates. However, if the extraction time is extended too much the yield slowly decreased due to insoluble material which is also being suspended in the solvent after rupturing of the cells, and it lowers the permeability of the solvent [1]. However, NaOH concentration and solid to liquid ratio had a complex relationship with the IDF. The concentration gradient between the solid and liquid is the driving force for mass transfer during solid-liquid extraction. When a higher solid/liquid ratio is used, the concentration gradient is larger, which improves mass transfer. However, as the solid to liquid ratio increased beyond a certain point, the extraction yield

decreased. The possible reason may be attributed to adding more liquid will not change the driving force because mass transfer is limited to the solid interior and thereby lowering dietary fiber recovery.

Likewise Fig.1(c) and (d) displayed the effect of independent variables on TDF. The Fig. 1(c), revealed that when the amplitude was enhanced to 35%, the TDF yield increased at first (up to 93.6%) and then progressively decreased to 56.21% with the amplitude at solid to liquid ratio of 1:20 and NaOH concentration of 1.2 mol/L. Fig. 1(d) showed that, the TDF yield increased with the NaOH solvent and decreased with the solid to liquid ratio at amplitude 30% for ultrasonication time of 30 min. However, for the increase in extraction time slight increase in TDF was noticed. The results of the present investigation were in line with the observation reported during extraction of soluble dietary fiber from papaya peel [37]. This could be attributed to the appropriate amplitude which destroyed the cell wall and leading to more yield of SDF. However, TDF might be degraded under high-intensity waves. The extraction time is also an important variable as TDF dissolution requires a certain time, but thermal degradation could occur if the extraction time is too long. The NaOH concentration and solid to liquid ratio had significantly affected the TDF yield. The TDF yield first increased to its maximum level as the NaOH concentration was increased to 1.0% and then decreased with further increase in the NaOH concentration. The results indicated that the NaOH treatment assisted by UAE, increased the fiber solubility or by weakening the strength of the bonds between fiber with cell wall constituents and increased the extraction rate [9]. TDF yield increased significantly up to 80% at solid to liquid ratio of 1:20 afterwards decreased at 30% amplitude for 30 min ultra-sonication treatment. Similar kind of observation was also reported by several authors. For instance, the polysaccharide was extracted from *L. japonica* using alkaline extraction [30], from *Orchis chusua D. Don* using ultrasonic-assisted extraction [28] and also from pineapple pomace using ultrasonic-cellulase synergistic extraction method [13] and all of these recorded the same increase and then revealed decreased trend with respect to these independent variables.

Table 2.1 Experimental design

Run	Time (min)	Amplitude (%)	Solid/Liquid	NaOH (mol/L)	IDF (%)	SDF (%)	TDF (%)
1	15	40	1:30	1	62.33	18.3	80.63
2	20	50	1:40	1.2	64.7	14.21	78.91
3	20	30	1:20	0.8	61.86	4.46	66.32
4	20	50	1:20	1.2	56.21	0.0001	56.21
5	20	50	1:40	0.8	74.07	8.74	82.81
6	20	30	1:40	0.8	64.6	13.27	77.87
7	20	30	1:20	1.2	63.59	7.58	71.17
8	20	30	1:40	1.2	56.37	25	81.37
9	20	50	1:20	0.8	53.31	5.35	58.66
10	25	40	1:50	1	72.49	9.8	82.29
11	25	40	1:30	1	58.91	13.88	72.79
12	25	40	1:10	1	69.19	0.55	69.74
13	25	60	1:30	1	57.56	7.21	64.77
14	25	40	1:30	1	57.12	15.12	72.24
15	25	20	1:30	1	52.74	5.73	58.47
16	25	40	1:30	1	60.29	13.12	73.41
17	25	40	1:30	1	61.67	15.63	77.3
18	25	40	1:30	1	59.19	13.72	72.91
19	25	40	1:30	1	58.91	11.88	70.79
20	25	40	1:30	1.4	64.56	19.02	83.58
21	25	40	1:30	0.6	60.82	7.38	68.2
22	30	50	1:20	1.2	71.22	9.9	81.12
23	30	30	1:40	0.8	53.96	3.96	57.92
24	30	50	1:20	0.8	56.15	8.89	65.04
25	30	50	1:40	1.2	57.45	12.37	69.82
26	30	30	1:20	1.2	81.9	11.7	93.6
27	30	30	1:40	1.2	60.92	14.63	75.55
28	30	50	1:40	0.8	56.86	7.35	64.21
29	30	30	1:20	0.8	61.38	5.94	67.32
30	35	40	1:30	1	57.69	17.7	75.39

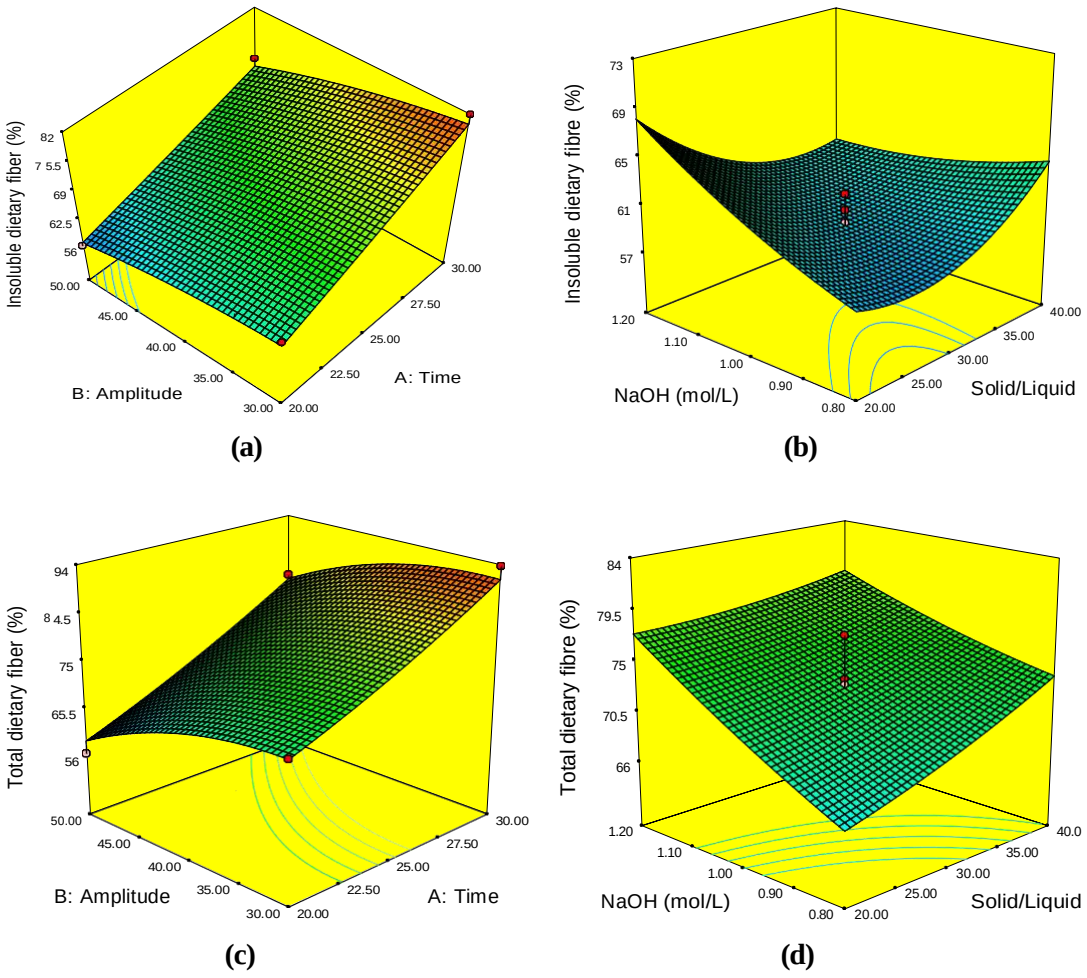


Fig 2.1 Response surface plot representing effect of (a) amplitude and time for IDF, (b) NaOH and solid:liquid for IDF , (c) amplitude and time for TDF, and (d) NaOH and solid :liquid for TDF

2.3.1.1 Optimization and validation

The ultrasound-assisted extraction of IDF in alkaline solution from pea peel was optimized based on highest desirability (0.87). ANOVA was performed to evaluate and screened the effects of significant variables in linear and quadratic forms (Table 2.1). The extraction process was optimized in terms of maximum IDF and maximum TDF. The optimized condition was; time 30 min, amplitude 30%, solid to liquid ratio (1:20), and NaOH concentration 1.2 mol/L. In the optimized condition the extraction was conducted to validate the extraction conditions and at optimized condition amount of IDF was 78.30% and SDF was 9.8%. Moreover, the experimental value did not vary significantly ($p \leq 0.05$) with the predicted value.

Table 2.2 Peleg's model parameters

Treatments	K_1 g_{db}/mg	K_2 g_{db}/mg	R^2	RMSE
ADF	0.007	0.011	0.99	2.017
UDF	0.012	0.013	0.98	2.817
ODF	0.009	0.012	0.99	1.975

Alkaline extraction (ADF), ultrasound assisted extraction (UDF) and ultrasound assisted alkaline extraction method (ODF).

2.3.2. Comparison of extraction kinetics

The influence of extraction methods on solid-liquid extraction yield is illustrated in Fig.2., where ADF (alkaline extraction), UDF (ultrasound assisted extraction) and ODF (ultrasound assisted alkaline extraction) are various extracted methods.

The results and extraction curves evinced the exponential growth of extraction rate initially for all the methods employed. The concentration of extracted IDF was highest during the ultrasonication in alkaline environment and the amount was 84.57%. The lowest IDF was observed for conventional extraction process and the amount was 75.82%. The modified Peleg's model (Eq.5) was adapted and fitted with experimental data. The calculated parameters of modified Peleg's model (constants K_1 and K_2), coefficient of determination (R^2) and RMSD are summarised and presented in Table 2.2. The coefficient of determination was high in all experiments ($R^2 \geq 0.98$) which implied good concordance between experimental and calculated data. Aguiló-Aguayo et al. [1] also reported similar observations during the extraction of polysaccharides from mushroom by-products. The highest values of extraction rate constant (K_1) and constant of extraction extent (K_2) were observed for the ODF as 0.012 and 0.013, respectively. The increase of extraction rate constant (K_1) and constant of extraction extent (K_2) yield might be due to the increase in the transfer rate form disruption of cells and structures owing to the ultrasonication in alkaline environment [1]. However, the lowest rate of extraction was observed for the conventional extraction process (Table 2.2). The RMSD had relatively low values and it justifies why the average deviation between the experimental and predicted value was minimum.

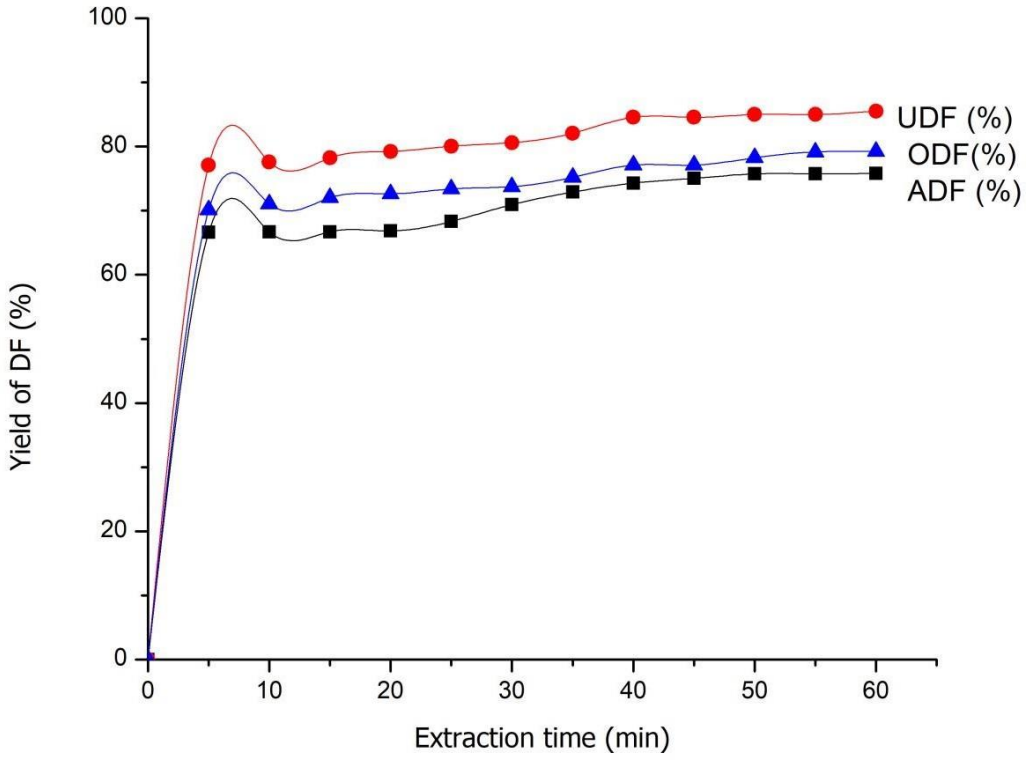


Fig 2.2 Extraction behaviour of insoluble dietary fiber in different methods

2.3.3. Physiochemical properties

The physicochemical properties of the dietary fiber extracted through different processes are presented in Table 2.3. There was a significant difference ($p \leq 0.05$) in the dietary fiber extraction through different extraction technique whereas least difference was found in their proximate analysis i.e., moisture, fat, protein, ash and carbohydrate. The structural changes caused by ultrasonic cavitation enhanced the extraction of IDF from the pea peel [1]. The IDF content was higher in UDF (79.69%) followed by ODF (63.92%) and ADF (55.72%), that could be due to the solvent used in UDF was water and in ODF the solvent was alkaline thus more impurities may be present in the UDF extraction where only ultrasound treatment was given. In alkaline medium, the interactive force between the SDF molecules becomes weaker and combination of ultrasound with alkali exhibited a synergistic effect in lowering the diffusion-limiting barriers between alkali and substrate.

Thereby its tissue becomes looser after ultrasound treatment which was not visible in

UDF treatment where alkali medium was not given [37, 31]. Therefore, SDF content in UDF was found to be negligible. In contrast, SDF content was significantly higher in other two extraction methods that is ADF and ODF wherein both cases alkali medium was given. Alkali treatment in combination with ultrasound might have destroyed the cell walls more significantly. Wang et al. [32] also mentioned that in the presence of alkaline solution hemicellulose in the IDF partially dissolved and converted the IDF into SDF, resulting in an increase in SDF content.

Table 2.3 Physiochemical properties of the dietary fiber

Properties	ADF	UDF	ODF
Moisture (g/100 g)	4.12 ± 0.31^{b}	4.01 ± 0.23^{b}	3.89 ± 0.61^{a}
Fat (g/100 g)	0.53 ± 0.02^{a}	0.61 ± 0.01^{b}	0.52 ± 0.01^{a}
Protein (g/100 g)	10.5 ± 0.92^{b}	8.02 ± 0.61^{a}	8.75 ± 0.39^{a}
Ash (g/100 g)	2.46 ± 0.36^{b}	2.89 ± 0.14^{c}	2.12 ± 0.51^{a}
Carbohydrate (g/100 g)	6.72 ± 0.98^{a}	6.81 ± 0.92^{a}	6.63 ± 1.34^{a}
L	58.45 ± 0.11^{a}	69.43 ± 0.28^{c}	63.55 ± 0.61^{b}
a	4.05 ± 0.20^{b}	0.58 ± 0.02^{a}	4.45 ± 0.68^{c}
b	24.15 ± 0.13^{b}	22.14 ± 0.51^{a}	56.79 ± 0.92^{c}
IDF (%)	55.72 ± 1.31^{a}	79.69 ± 3.2^{c}	63.92 ± 2.64^{b}
SDF (%)	17.41 ± 1.92^{c}	0.99 ± 0.1^{a}	14.00 ± 0.87^{b}
TDF (%)	73.13 ± 2.14^{a}	80.68 ± 3.21^{c}	77.92 ± 3.51^{b}

Different superscripts in the same row represents the significant difference at $p\leq0.05$. Alkaline extraction (ADF), ultrasound assisted extraction (UDF) and ultrasound assisted alkaline extraction method (ODF).

2.3.4. Functional properties

The functional properties of the GPP dietary fiber are significantly affected by the extraction processes and are summarized in the Table 2.4. Water holding capacity (WHC) represents the capacity of the moist material in water retention when an external compression or centrifugal gravity force is applied, such as the physically trapped water, hydrodynamic water and linked water. The highest WHC (0.127 g water/g dry sample) of dietary fiber was observed for the ODF extracted sample. The WHC is associated with the hydrophilic site chemical nature and quantity, as well as diverse dietary fiber surface areas, densities and structures [3]. Therefore, due to ultrasonication in presence of alkaline, there might be significant breakdown in the microstructure as well as increase in the hydrophilic site in the dietary fiber [32]. The dietary fiber extracted by ODF

demonstrated high oil holding capacity (OHC). Basically, the mechanism of OHC is mainly due to the physical entrapment of oil by capillary attraction [34]. Therefore, due to the ODF treatment, there might be destruction or enlargement of microstructural pore, which might reduce the OHC of the dietary fiber. Typically, the high WHC and low OHC indicate that dietary fiber samples extracted from pea peel might be a good dietary resource for developing related functional food products, which can help to avoid water syneresis in formulated foods and act as the emulsifier for foods with a higher fat content [33].

Table 2.4 Functional and thermal properties of ADF, UDF and ODF dietary fiber

	Functional properties			**Thermal properties**		
Treatments	WHC (g water/g dry sample)	OHC (g oil/g dry sample)	SC (mL/g dry sample)	**To (°C)**	**Tp (°C)**	**Tc (°C)**
ADF	0.061 ± 0.001^{a}	2.68 ± 0.25^{b}	9.5 ± 0.48^{a}	101.7	124.3	153.8
UDF	0.059 ± 0.01^{a}	2.00 ± 0.1^{a}	11.0 ± 0.92^{c}	102.6	121.4	155.7
ODF	0.127 ± 0.01^{b}	2.92 ± 0.20^{c}	10.5 ± 0.46^{b}	101.0	118.4	149.0

Different superscripts in the same row represents the significant difference at $p\leq0.05$. Alkaline extraction (ADF), ultrasound assisted extraction (UDF) and ultrasound assisted alkaline extraction method (ODF).

2.3.5. FT-IR analysis of extracted IDF from GPP

The FT-IR spectra of dietary fiber extracted through ADF, UDF and ODF are illustrated in Fig 2.3. The significant peaks observed in the wavenumber range of 3600-2900 cm^{-1} are having notable features for stretching vibration in polysaccharide for O-H and C-H bonds [12]. An extensive, tensile peak resembles the O-H stretching of hydrogen atom attached to hydroxyl group at 3463 cm^{-1} and a minor peak was detected at 2907 cm^{-1} which shows C-H stretching of methylene group in polysaccharide [2]. Thus, these peaks also included the structure of intra and inter-molecular hydrogen bonds in cellulose and hemicelluloses [12]. The absorption spectrum at 1415 cm^{-1} belongs to the bending and stretching vibrations of CH_2, where the aromaticity of lignin structure was confirmed by aromatic hydrocarbon peaks at 1630 cm^{-1}. However, the peaks found at 1027 cm^{-1} correlated to C-O bond stretching which indicated the deformation of oligosaccharides from IDF. Appearance of all these bands vibration confirmed the typical polysaccharide structure of ADF, UDF as well as in optimised ODF.

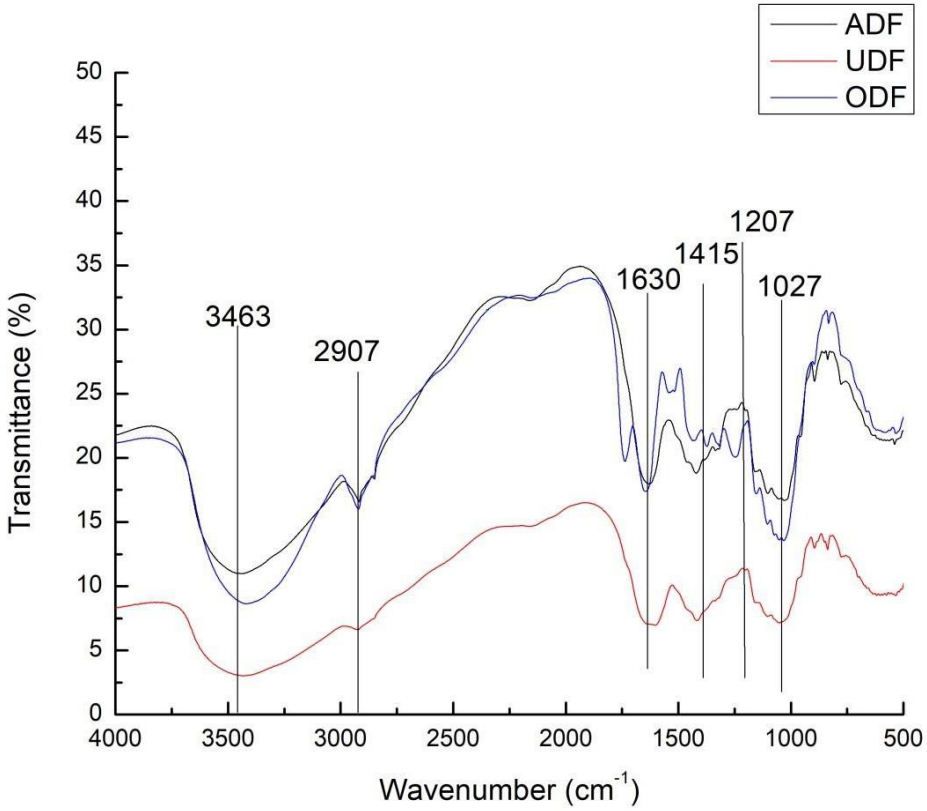


Fig 2.3 FT-IR spectrum of ADF, UDF and ODF

2.3.6. Thermal properties

The thermal properties of GPP dietary fiber extracted through various methods in terms of onset, peak and conclusion temperature are presented in Table 2.4. The ODF revealed lowest thermal stability in terms of peak temperature (118.4°C) and conclusion temperature (149.0°C). In the DSC curve, the exothermic peak corresponds to thermal and oxidative decomposition of the polymer and the vaporization with elimination of volatile products. The pyrolysis of polysaccharides is initiated by the random breakdown of glycosidic bonds followed by further decomposition [25].

2.3.7. Thermal decomposition

The thermogravimetric parameters mainly associated with weight loss and degradation temperature of fibers is presented in Fig 2.4. Thermal stability is an important property of a dietary fiber when it is thermally processed in food processing industry [27]. It could be seen from the Fig 2.4 that the extraction processes have significant effect on the IDF.

The highest mass loss of IDF was observed for the UDF that was 57.75% and lowest mass loss was observed for the ODF as 44.76% at 425°C. For the ultrasound treatment, inter and intra molecular interactions of IDF might breakdown and thereby enhances the release of moisture from the structure. However, during ultrasound in presence of alkaline (ODF), the IDF might breakdown in small size which could retain the moisture in structure. The highest residual mass was observed for the ODF (41.82%) followed by ADF (36.20%) and UDF (33.82%). UDF had significantly least thermal stability than other treatments, which demonstrated a rapid weight loss of 57.75% at 425°C. Results suggested that ODF had the superior thermal stability that could be due to the presence of alkaline medium with ultra-sonication extraction treatment which allowed the surface to break and increased its hydrophilic site [33].

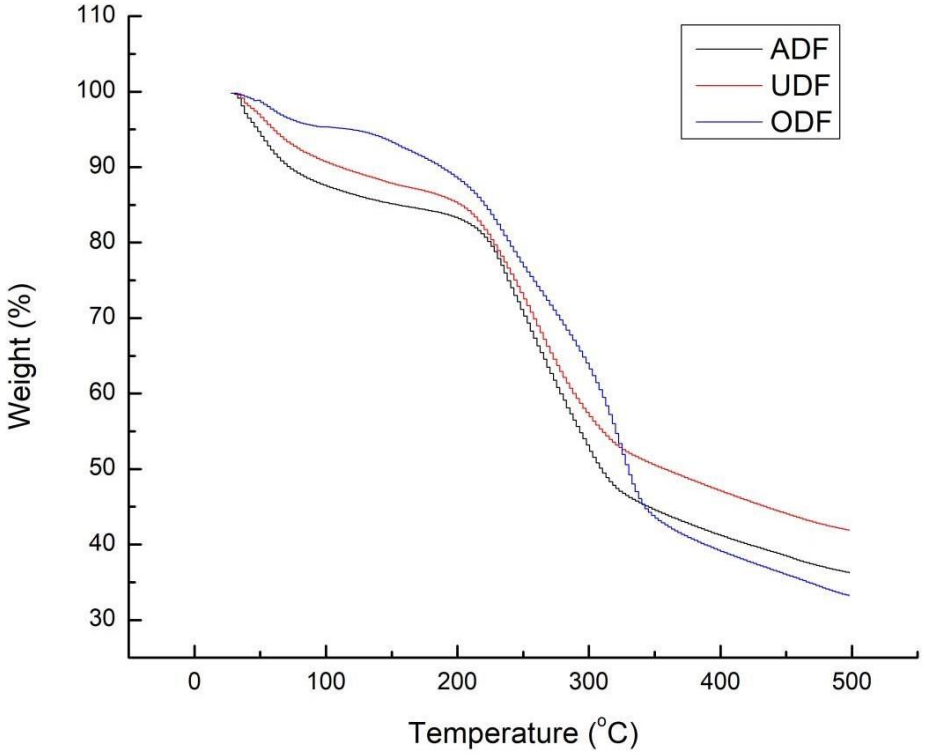
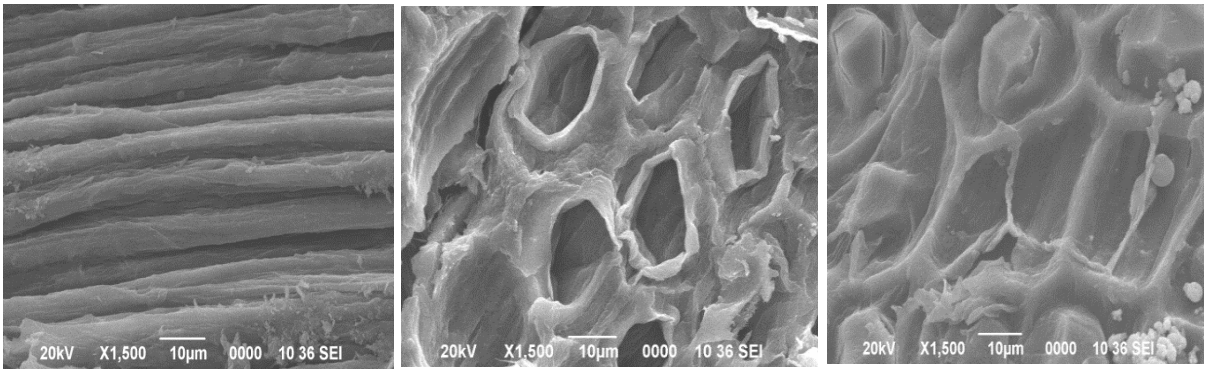


Fig 2.4 Thermal decomposition of extracted ADF, UDF and ODF

2.3.8. Morphology

The morphological properties of extracted IDF using different methods showed different morphological properties (Fig 2.5). IDF was extracted by alkaline treatment where the

microstructure of dietary fiber had demarcated compact long matrix like structure and the pores were relatively negligible. Long thread like network was visible in the alkaline condition as shown in Fig. 5(a). Cellulose, hemicellulose, and lignin are the main building materials of cell wall creating microfibrils and all these compounds interlace to several layers together to form the cell wall of DF [21]. In contrast, ultrasound treatment disrupted the crosslink between polysaccharides, deformed parenchyma cells and disintegrated the cellulose effectively. Its morphology had looser structure and pore expansion due to ultrasonication and resulted in honeycomb structure with deeper cavities or spaces observed as represented in Fig 2.5(b) [8, 32]. Similarly, ultrasound assisted with alkaline treatment also effectively changed the morphology of the extracted IDF shown in Fig 2.5(c). Pores in the cell wall become looser and cavities also expanded forming larger specific surface area which might have been fairly entrapped the water molecules and thus enhances the water holding property [11].



(a) **(b)** **(c)**

Fig 2.5 Surface morphology of extracted (a) ADF, (b) UDF, and (c) ODF

2.4. Conclusions

The present study explicated that alkaline extraction (ADF), ultrasound assisted extraction (UDF) and ultrasound assisted alkaline extraction (ODF) methods on GPP dietary fiber evinced changes in the yield, kinetics, thermal stability, morphology, and the functional properties of IDF. The yield of the IDF was much higher in UDF and ODF compared to ADF. The thermal degradation was lowest for ODF, in terms of mass loss over the other extraction processes. The ODF showed lowest thermal stability in terms of peak temperature (118.4°C) and conclusion temperature (149.0°C). Moreover, the morphology of ODF showed typical honeycomb structure, which made larger pores in

the cell wall and loosed the fibrous structure and enhanced the functionality. The ODF was found to be the best extraction method with rich biological potential and higher yield. Characterization of IDF of GPP revealed that this fiber might be considered as the potential element which could be used as one of the functional ingredients in dairy, meat industries, beverages such as snacks, pasta and ready to eat cereals to provide stability, emulsion strength and water holding capacity. This potential application could create substantial economic benefits by utilizing this GPP by-product and thereby lessen the environmental footprint.

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