Detailed Technical Progress Report

Approved Objectives:

1. Comparative biochemical, phytochemical and physiochemical study of inner and outer bracts of culinary banana flower
2. Optimization of soluble and insoluble fiber extraction from culinary banana flower using conventional (mechanical) and combined (mechanical & ultrasonic) treatment based on RSM
3. Toxicity studies of dietary fiber concentrate
4. Incorporation of antioxidant rich dietary fiber concentrates into food model and sensory evaluation
5. Preparation of biopolymer based nanocellulose fiber and its application in film
6. Developed product to be tested among the local population for acceptance

Works Done:

Objective 1: Comparative biochemical, phytochemical and physiochemical study of inner and outer bracts of culinary banana flower

Materials and methods

1.1. Procurement of chemicals and raw materials

All chemicals were purchased from Merck Millipore, Himedia, SRL and Sigma Aldrich Corporation, USA. All the chemicals and reagents were of high purity analytical grade. The optimum stage of harvesting banana flower samples is considered to be 50 days (Khawas, 2016). Six different samples of banana flower were collected at the optimum stage of harvesting from six different local areas of Napaam, Tezpur, Assam. All the samples required for analysis were procured at one time. Samples were washed immediately after collection and outer and inner bracts were separated and dried separately at 40°C in a tray drier (Labotech, BDI-51, India). Samples were ground, packed in air tight container and stored at room temperature (25±5°C) until further analysis.

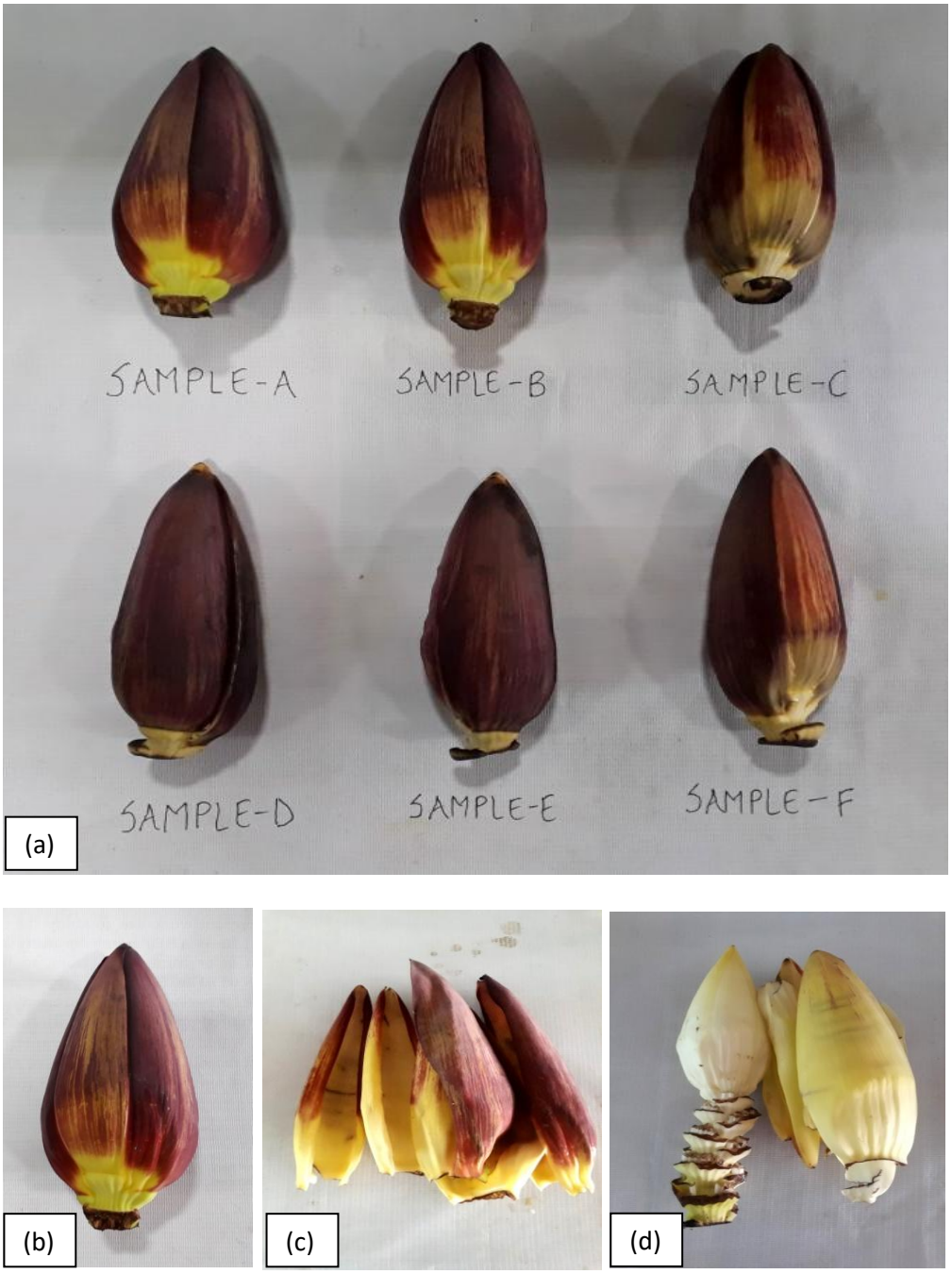


Fig.1.1 (a) Six different culinary banana (*Musa* ABB) flower samples collected from six different local areas of Napaam, Tezpur, Assam; (b) Whole Culinary banana flower; (c) Culinary banana flower outer bracts; (d) Culinary banana flower inner bracts

1.2. Proximate Analysis of Outer and Inner Bracts of Culinary Banana Flower

1.2.1. Moisture content (AOAC, 2010)

The moisture content of both the fresh and ground powder of the inner and outer bracts of culinary banana flower (*Musa* ABB) was determined by oven drying. This involved determination of the difference in weight before and after drying expressed as a percentage of the initial powder weight.

$$\text{Moisture content \%} = \frac{b-c}{a} \times 100$$

Where, a - Weight of the sample

b - Weight of the dish with sample before drying

c - Weight of dish with sample after drying

1.2.2. Ash content (AOAC, 2010)

Powdered sample of 2 g was taken in a crucible and kept inside a muffle furnace at 600°C till white ash is obtained (4 h). After ashing, the crucibles were kept in a desiccator to bring down the temperature and weight was taken. Total ash content was calculated using the formula given below.

$$\text{Total ash content \%} = \frac{a-b}{C} \times 100$$

Where, a - Weight of crucible with sample after ashing

b - Weight of empty crucible

c - Weight of sample

1.2.3. Crude fiber estimation (AOAC, 2010)

Dried sample of 2 g was taken and digested/boiled with 200 ml of 1.25% H_2SO_4 for 30 min. After 30 min the solution was cooled down and filtered with muslin cloth. The residue was washed with hot water to remove acidity. The residue was then transferred to conical flask containing 200 ml of 1.25% NaOH and boiled for 30 min at 400ºC. The solution was then cooled and filtered. The remaining residue i.e., fiber was then transferred to crucible. The residue was then washed with 15 ml alcohol and kept under hot air oven for 24 h at 130ºC. After weighing of the sample, it was then taken to furnace at 600ºC for 30 min and left for cooling and again weighed it.

$$\text{Crude fiber content (\%)} = \left(\frac{\text{crude after ignition}}{\text{wt.of sample}}\right) \times 100\%$$

1.2.4. Crude fat estimation (AOAC, 2010)

Fat content was measured using solvent extractor apparatus. The empty weight of pre dried flask was taken. The solvent used is petroleum ether (boiling point 60-80ºC). Powdered sample of 2 g was weighed and packed in filter paper. It was then placed inside the flask. This flask was then placed in a Soxhlet apparatus and run at 80ºC for 4 h. After completion, the solvent present evaporated completely. The oil containing flask was then weighed and fat was calculated using the following formula:

$$\text{Fat} = \frac{a-b}{c} \times 100$$

Where, a- Weight of round bottom flask containing lipid

b- Empty weight of round bottom flask

c- Weight of sample

1.2.5. Protein estimation

The protein estimation of the sample was done by the method adopted by Lowry *et al.,* 1951. The sample extract of 0.1 ml and 0.2 ml were pipette out in two different test tubes and the volume was made up to 1 ml with water. A tube with 1 ml of water was served as blank. 5 ml of alkaline copper solution was added and mixed well which was then incubated at room temperature for 10 min. 0.5 ml of FCR was added and mixed well immediately followed by 30 min incubation at room temperature. Finally, the absorbance was read at 660 nm against blank and the amount of protein in the sample was calculated.

1.3. Other Biochemical Analysis

Insoluble, soluble and total dietary fibres were determined according to enzymatic-gravimetric method (Prosky et al., 1988). **Cellulose and hemicellulose content** were determined according to Updegroff (1969). **Pectin content** was determined as calcium pectate (Ranganna, 2008).

1.4. Analysis of total polyphenol content and antioxidant activity

The outer and inner bracts of culinary banana flower were ground with 80% and were placed in an ultrasonic bath (Bandelin Sonorex, Germany) with frequency of 35 kHz for 15 min. The extracted samples were filtered through Whatmann No. 1 filter paper and kept at 4°C in the dark until further analysis.

1.4.1. Estimation of total polyphenols

Total polyphenols content were determined spectrophotometrically at 650 nm using Folin- Ciocalteau reagent with catechol as a standard (Bray & Thrope, 1954). Exactly 0.5 to 1 g of the sample was weighed and mixed with 10 times volume of 80% ethanol. The homogenate was centrifuged at 10000 rpm for 20 min. The supernatant was saved. The residue was re-extracted with five times the volume of 80% ethanol, centrifuged, and pooled the supernatants. The supernatant was evaporated to dryness and dissolve the residue in a known volume of distilled water (5 ml). Different aliquots (0.2 to 2 ml) were allowed to pipette out into test tubes. The volume in each test tube was made up to 3 ml with water and added with 0.5 ml of Folin-Ciocalteu reagent. After 3 min, 2 ml of 20% Sodium carbonate solution was added to each tube. It was mixed thoroughly and the tubes were placed in a boiling water bath for exactly 1 min, cooled and the absorbance was measured at 650 nm against a reagent blank. A standard curve was prepared using different concentrations of catechol. **Calculation:** From the standard curve, the concentration of phenols in the test sample was found out and expressed as mg phenol/100 mg material.

1.4.2. Antioxidant activity determination

1.4.2.1. DPPH free radical scavenging activity

DPPH free radical scavenging activity was determined according to the methods of (Brand-Williams et al., 1995) with some modifications. A stock solution was prepared by dissolving 24 mg DPPH in 100 ml methanol. The working solution was prepared by diluting the stock solution until the absorbance of 1.1±0.01 units at 515 nm obtained. Extract from outer and inner bract (150 µl) were allowed to react with DPPH solution (2850 µl). After 30 min, the absorbance was taken at 515 nm. The standard curve was prepared with Trolox, concentration ranging from 25 to 800 µM. Results were expressed in µM Trolox equivalents (TE)/g fresh mass. The IC_{50} for different concentration of outer and inner bract were determined and compared with standard.

1.4.2.2. ABTS assay

The ABTS assay was performed according to the method of Arnao et al. (2001) with some modifications. $ABTS^{+}$ solution (7.4 mM) and potassium persulfate solution (2.6 mM) was prepared. Working solution was prepared by mixing equal amount of the above solutions and allowed them to react for 12 h in dark. The solution was diluted with methanol to obtain an absorbance of 1.1±0.01 units at 734 nm. Extract of 150 µl each from outer and inner bracts were allowed to react with 2850 µl of working solution for 2 h. The absorbance was taken at 734 nm. The standard curve was prepared with Trolox, concentration

ranging from 25 to 600 µM. Results were expressed in µM Trolox equivalents (TE)/g fresh mass. The IC_{50} for different concentration of outer and inner bract were determined and compared with standard.

1.4.2.3. FRAP assay

FRAP assay was done according to the method of Benzie & Strain (1996) with some modifications. The working solution was prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10 mM) and 2.5 ml $FeCl_3$. $6H_2O$ solution (20 mM) and warmed it at 37°C. Extract of 150 µl each from outer and inner bracts were allowed to react with 2850 µl of FRAP solution for 30 min. The absorbance was taken at 593 nm. The standard curve was prepared with Trolox, concentration ranging from 25 to 800 µM. Results were expressed in µM Trolox equivalents (TE) /g fresh mass.

1.5. Mineral contents

The mineral contents were estimated by atomic absorption spectrophotometer (Thermo Scientific, ICE 3000 Series, Newington, USA). Powder samples (0.5 g) were added to 15 ml concentrated nitric acid and perchloric acid in the ratio of 2:3. Samples were digested in a digester (KelPlus, Pelican Equipment, India). At the end of the digestion process, digests were cooled and diluted up to 100 ml with distilled water. Concentrations were determined in the aqueous solution of acid digest.

1.6. HPLC analysis of phenolic extract

Extraction of free and bound phenolics was done according to the method described by Okarter et al. (2010) with some modifications.

For free phenolic extraction, the outer and inner bracts powder were defatted with n hexane (bp 70°C) and extracted with 80% methanol at room temperature (25±5°C) and sonicated using ultrasonic bath (Bandelin sonorex, Germany) at 35 kHz for 15 min and the extraction continued until the solvent became colourless. The extracts were filtered through Whatman No. 1 filter paper and the solvent was removed using rotary vacuum evaporator (EYELA, NCB-1200, Tokyo) at 40°C and stored under refrigeration until further analysis.

Bound phenolics were extracted from the 126 residue of free phenolics. The residues of outer and inner bracts were hydrolyzed with 2M NaOH under nitrogen atmosphere for 1 h. The pH was adjusted to 2.0 and then phenolics were extracted with ethyl acetate. It was then evaporated to dryness using rotary vacuum evaporator at 40°C and dissolved in 10 ml methanol and stored under refrigeration for analysis.

The HPLC analysis was conducted following the method of Rodriguez-Delgado et al. (2001) with some modifications. Detection was done using HPLC system (Dionex, Ultimate 3000, Germany). The column used was Acclaim 120-C18 column (5 µm, 120 Å) with size of 4.6×250 mm. A mixture of

methanol:acetic acid:water (10:2:88, v/v) and methanol:acetic acid:water (90:2:8, v/v) were used as solvent A and solvent B, respectively. The gradient program was 0-15 min 15% B, 16-20 min 50% B, 21-35 min 70% B, 36-50 min 100% B and 51-60 min 15% B. The injection volume was 20 µl, and the flow rate was 1 ml/min. Detection was done at 280 nm and sample peaks were identified by comparing with retention times of standard peaks.

1.7. Functional properties

Bulk density of the sample was determined by the method of Wang & Kinsella (1976). Powdered sample (3 g) was placed in a 50 ml graduated cylinder and packed by tapping the cylinder on a rubber sheet until a constant volume was obtained and the bulk density was expressed as g of sample/ml.

To determine water holding capacity (WHC) or oil holding capacity (OHC) of the banana bracts and cellulose, 25 ml of distilled water or olive oil were added to 250 mg of dry sample followed by stirring and incubation at room temperature (25±5°C) for 1 h. Tubes were centrifuged at 3000 g for 20 min and the supernatant was decanted and tubes were allowed to drain for 10 min to ensure the proper removal of water or oil but not the residue. The residue was weighed and WHC or OHC was calculated as g water or oil per g dry sample, respectively as per the method described by Rodriguez et al. (2006) with slight modification. To determine water swelling capacity (WSC), samples (200 mg) were mixed with 10 ml distilled water in calibrated cylinders and incubated at room temperature (25±5°C) for 18 h. The bed volume was recorded. The WSC was determined as ml per g of dry sample following the method described by Robertson et al. (2000).

Glucose dialysis retardation index (GDRI) of outer and inner bracts, and cellulose was determined according to the method described by Lecumberri et al. (2007). Outer bracts, inner bracts and cellulose (400 mg each) were extracted with 80% ethanol to ensure the total removal of soluble sugar and mixed with 15 ml of distilled water followed by constant stirring for 1 h at room temperature (25±5°C). An equal amount of glucose solution (2 mg/ml) was added to both samples before transferring to 15 cm portions of previously hydrated dialysis bags. An equal volume of glucose solution without sample was placed in a dialysis bag and considered as control. Samples were dialyzed against 400 ml of distilled water in separate beakers and incubated in a shaking water bath at 37°C for 60 min. After 20, 40 and 60 min, 0.5 ml of dialysate were collected from each sample and glucose concentration was determined using anthrone method (Sadasivam & Manickam, 1992). The retardation of glucose diffusion from the dialysis bag into the dialysate was calculated as below:

$$\text{GDRI} = 100 - \left(\frac{total\ glucose\ diffusion\ from\ sample}{total\ glucose\ diffusion\ from\ control}\right) \times 100$$

Results and discussion

The chemical compositions of the outer and inner bracts of culinary banana flower are presented in Table 1.1, Table 1.2, Table 1.3 and Table 1.4. DF was the major component in both outer and inner bracts among all the components. The insoluble DF was found to be higher compared to soluble DF for both outer and inner bracts. The outer and inner bracts contained higher total DF of 61.00% and 66.18%, respectively in the six different samples. The results are in line with the findings of Bhaskar et al. (2012) who reported the DF content of banana (*Musa* sp. var. elakki bale) flower as 65.6%. Ma and Mu (2016) reported total DF content of rice bran (27.04%), peach DF concentrate (30.7%), oranges DF (35.4-36.9%) and sesame coat DF concentrate (31.64%). Hence, the total DF content of outer and inner bracts of culinary banana flower was higher than several fibre-rich foods. Different components of DF viz.., cellulose, hemicellulose and pectin contents were also analysed and are shown in Table 1.3 and Table 1.4.

Table 1.1 Proximate Analysis of the outer bract powder samples of six culinary banana flowers collected from six different areas

Component (g/100g)	Outer bract					
	S_1	S_2	S_3	S_4	S_5	S_6
Moisture content	8.47	8.82	8.32	7.95	8.35	8.14
Crude fat	4.78	3.8	4.1	3.8	4.6	4.3
Protein	2.06	2.14	2.32	2.31	2.47	2.19
Crude fibre	9.52	9.78	8.59	8.82	9.55	8.91
Ash	14	13.1	12.87	13.95	13.34	13.98
Starch	7.78	7.24	7.55	7.74	7.31	7.22

Table 1.2 Proximate Analysis of the inner bract powder samples of six culinary banana flowers collected from six different areas

Component (g/100g)	Inner bract					
	S_1	S_2	S_3	S_4	S_5	S_6
Moisture content	8.44	7.87	7.88	8.16	8.24	8.17
Crude fat	7.92	7.83	7.76	7.94	7.87	7.69
Protein	2.55	3.17	2.67	2.81	2.81	2.58
Crude fibre	10.73	10.24	10.37	10.64	10.66	10.41
Ash	10.1	10.52	10.21	10.41	10.11	10.32
Starch	16.8	16.24	16.57	16.44	16.35	16.49

Table 1.3 Other Biochemical Analysis of the outer bract powder samples of six culinary banana flowers collected from six different areas

Component (g/100g)	Outer bract					
	S_1	S_2	S_3	S_4	S_5	S_6
Insoluble Dietary fibre	53.9	53.21	52.91	53.49	52.97	53.64
Soluble dietary fibre	7.23	7.17	7.23	7.16	6.95	6.89
Total dietary fibre	61.13	61.11	60.87	61.1	60.95	60.84
Cellulose	5.47	5.46	5.32	5.45	5.39	5.82
Hemicellulose	18.83	18.81	18.67	18.74	18.77	18.72
Pectin as calcium pectate	5.31	5.3	5.19	5.29	5.23	5.28
Total polyphenol content (mg phenols/g dry sample)	7.56	7.47	7.42	7.29	7.54	7.51

Table 1.4 Other Biochemical Analysis of the inner bract powder samples of six culinary banana flowers collected from six different areas

Component (g/100g)	Inner bract					
	S_1	S_2	S_3	S_4	S_5	S_6
Insoluble Dietary fibre	61.86	61.79	61.59	61.77	61.59	61.84
Soluble dietary fibre	4.36	4.18	4.32	4.35	4.25	4.21
Total dietary fibre	66.22	66.19	66.14	66.21	66.17	66.12
Cellulose	13.19	13.18	12.95	12.97	13.15	12.91
Hemicellulose	14.36	14.27	14.11	14.28	14.24	14.35
Pectin as calcium pectate	3.97	3.91	3.95	3.85	3.67	3.82
Total polyphenol content (mg phenols/g dry sample)	9.44	9.39	9.37	9.43	9.41	9.37

The mineral content viz., K, Ca, Mg, and Na of outer bracts was significantly higher compared to inner bracts, among which K content was the highest (Table 1.5 and Table 1.6). The micronutrients viz., Fe, Cu and Zn were also found higher in outer bracts compared to inner one. Sheng et al. (2010) also reported banana flower (cvs. *Baxijiao* (AAA) and *Paradisica* (AAB)) as a good source of minerals viz., Mg, Fe, Cu and K.

Table 1.5 Mineral profiles of outer bract powder samples of six culinary banana flowers collected from six different areas

Minerals (mg/100g)	Outer Bract					
	S_1	S_2	S_3	S_4	S_5	S_6
Ca	651.86	648.71	649.10	648.16	650.32	646.91
Mg	567.56	565.45	568.11	567.41	567.24	566.19
Na	61.26	62.42	60.19	60.45	62.78	61.01
Fe	26.99	26.15	26.47	27.01	27.12	26.87
Zn	28.62	28.29	29.11	28.59	28.50	29.07
Cu	79.59	79.62	79.01	78.97	80.01	80.23
K	1800.01	1801.20	1809.01	1817.8	1819.34	1800.71

Table 1.6 Mineral profiles of inner bract powder samples of six culinary banana flowers collected from six different areas

Minerals (mg/100g)	Inner Bract					
	S_1	S_2	S_3	S_4	S_5	S_6
Ca	317.83	318.14	318.29	317.85	317.67	317.55
Mg	305.09	304.57	304.49	305.14	305.21	305.07
Na	17.94	17.68	17.88	18.07	17.92	18.02
Fe	14.51	14.13	14.47	14.53	14.20	15.09
Zn	9.13	9.11	8.98	8.76	9.22	9.14
Cu	5.53	5.15	6.03	5.62	6.12	6.10
K	1087.92	1088.10	1087.15	1087.29	1088.00	1087.75

The chemical compositional analysis showed that culinary banana bracts are rich source of nutrients and contains significant amount of minerals (Table 1.1, Table 1.2, Table 1.3, Table 1.4, Table 1.5 and Table 1.6). Incorporation of fibre-rich bracts into a wide range of products has the potential to contribute to the development of functional foods that are currently in high demand. Furthermore, the bracts of culinary banana flower could be commercially utilized for extraction of DF and utilization of it in food product will unleash a new source of fibre coupled with nutritional and dietary fibre requirements in a ready-to-eat form. Apart from this, bracts of culinary banana yields considerable amount of cellulose and water-soluble polysaccharide (WSP) which could also be a good source of bio-based packaging

material and therefore utilization of it will give high value to the agricultural waste of culinary banana bracts.

The antioxidant activity was determined by DPPH, ABTS and FRAP assay (Table 1.7). The percent inhibition of DPPH free radical and ABTS radical of inner and outer bracts are illustrated in Fig. 1.2. Both inner and outer bracts potentially scavenged DPPH and ABTS radical and it increased in a dose dependent manner (Fig. 1.2). The per cent inhibition of DPPH free radical and ABTS radical of inner bract increased much faster by increasing the concentration followed by outer bract and are very close to the positive control (Trolox). The concentration at 50% inhibition (IC_{50}) of DPPH free radical and ABTS radical was determined from the standard curve (Fig. 1.2). The IC_{50} values of DPPH free radical scavenging activity and ABTS radical scavenging activity (Table 1.7) revealed that inner bracts showed greater antioxidant activity (27.96 and 24.53 µmol TE/g fresh mass of inner and outer bract, respectively) than outer bracts with lesser IC_{50} value (163.50 and 219.06 µM of inner and outer bracts, respectively) for DPPH assay. The IC_{50} value of Trolox was 105.66 µM and similar results were observed for ABTS assay with lower IC_{50} value (241.83 and 296.46 µM of inner and outer bracts, respectively) in inner bracts when compared to the outer bracts. The lower IC_{50} value indicates higher scavenging free radical activity of an antioxidant (Zhang et al., 2015) and higher antioxidant activity for inner bracts (20.6, 8.85 µmol TE/g fresh mass of inner and outer bract, respectively) was also evidenced by FRAP assay.

Table 1.7 Antioxidant activity of outer and inner bracts of culinary banana flower

	DPPH		ABTS		FRAP
	Antioxidant activity (µmol TE/g fresh mass)	IC_{50}(µM)	Antioxidant activity (µmol TE/g fresh mass)	IC_{50}(µM)	Antioxidant activity (µmol TE/g fresh mass)
Outer bract	24.53	219.06	29.62	296.46	8.85
Inner bract	27.96	163.50	30.66	241.83	20.6
Positive control (Trolox)		105.66		148.60	

IC_{50}: Concentration at 50% radical scavenging activity

The DPPH assay of antioxidant is based on the activity of quenching free radicals or H-donor capability of the antioxidant. Increased concentration of the extracts of inner and outer bracts resulted in enhancement of the scavenging activity of DPPH free radicals. This indicated the increased ability of the compound present in the extracts to donate hydrogen ions. Zhang et al. (2015) reported that the IC_{50} value for DPPH free radical scavenging activity of different natural and synthetic standards such as propyl

gallate (PG), gallic acid (GA), quercetin (QCT) and Trolox were 35.5 ± 2.8, 36.3 ± 2.0, 55.0 ± 2.7 and 128.3 ± 6.3 µmol/l, respectively. The IC_{50} of butylated hydroxytoluene (BHT) was over 1000 µmol/l.

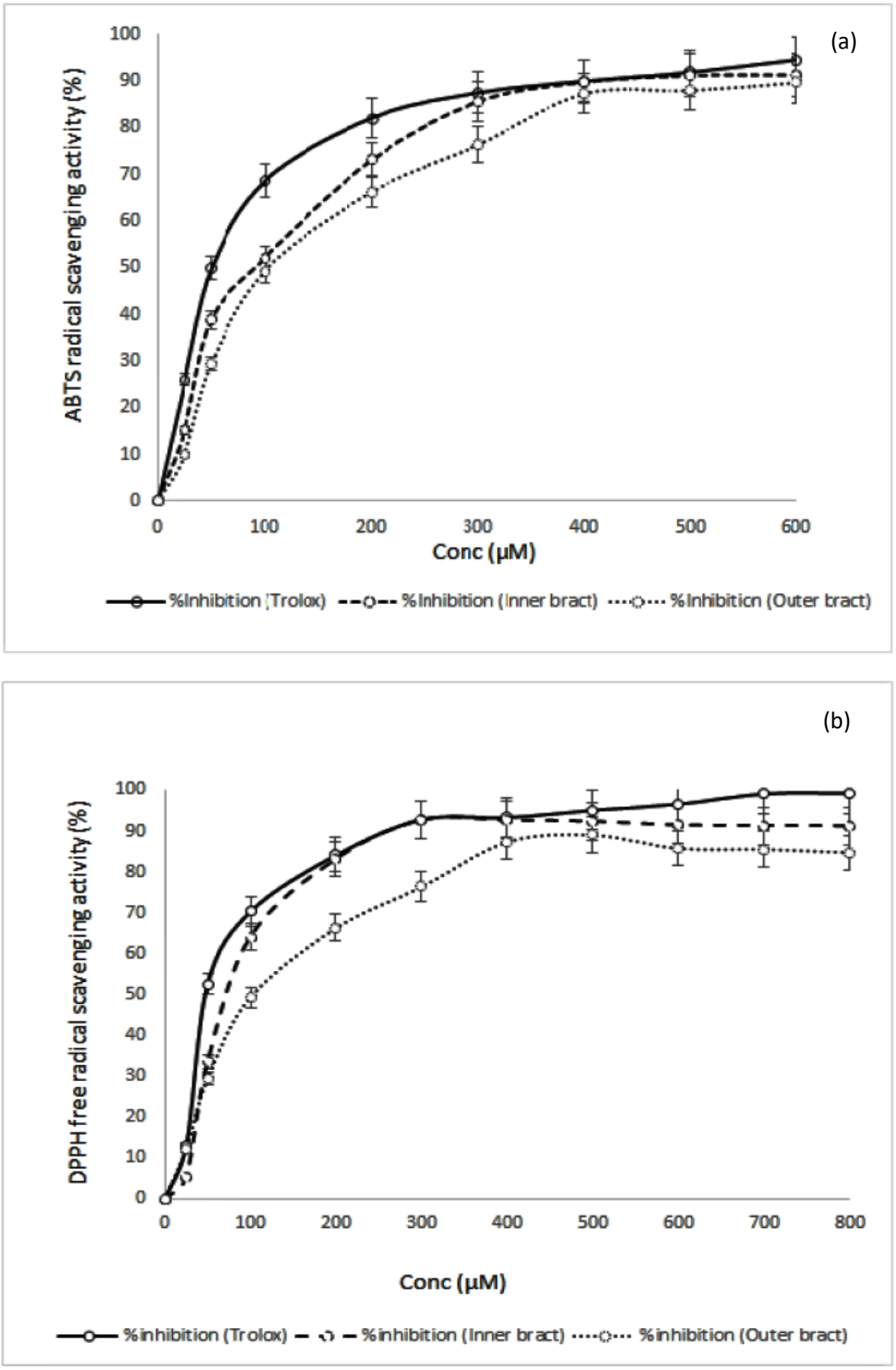


Fig.1.2 (a) DPPH free radical scavenging activity and (b) ABTS radical scavenging activity of Trolox, inner and outer bracts of culinary banana flower at varying concentration

Furthermore, the inner and outer bracts evinced good ability to scavenge the ABTS radical. FRAP assay is a singlet electron transformer method which is based on the reduction capability of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) of a test sample. Du et al. (2014) reported that the reduction capability of miracle berry (*Synsepalum dulcificum*) flesh extract was 22.9 mmol/100 g. The higher reduction capability attributed to the higher levels of phenolics and ascorbic acid, which is considered to be stronger reductants in donating electrons. The average antioxidant activity of methanolic extracts of different varieties of guava (white fleshed "Allahabad Safeda", pink-fleshed "Fan Retief", "Ruby Supreme", "Advanced Selection") as determined by ABTS, DPPH and FRAP assays were 31.1, 25.2 and 26.1 μM TE/g, respectively. The antioxidant activity of 12 fresh fruits (melon, pear, tomato, apple, banana, white and pink grape, pink grapefruit, orange, kiwi, plum, strawberry) ranged from less than 1 μM TE/g for melon up to 15 μM TE/g for strawberry (Thaipong et al., 2006). The antioxidant activity of inner and outer bracts was found to be very close to that of different guava varieties. This exceptionally high antioxidant activity of bracts extract as obtained by three different antioxidant (DPPH, ABTS and FRAP) assays might be due to the pronounced total phenolic content and phenolic compounds present in the bracts extract, which were evidenced by HPLC analysis. Sheng et al. (2010) reported banana flowers (cvs. *Baxijiao* (AAA) and *Paradisiaca* (AAB)) are good sources of antioxidants including phenolics and flavonoids. The ethyl acetate and methanol extract of *Musa paradisiaca* (Nendran variety) inflorescence also showed significant ABTS radical scavenging activity (Aruna et al., 2017).

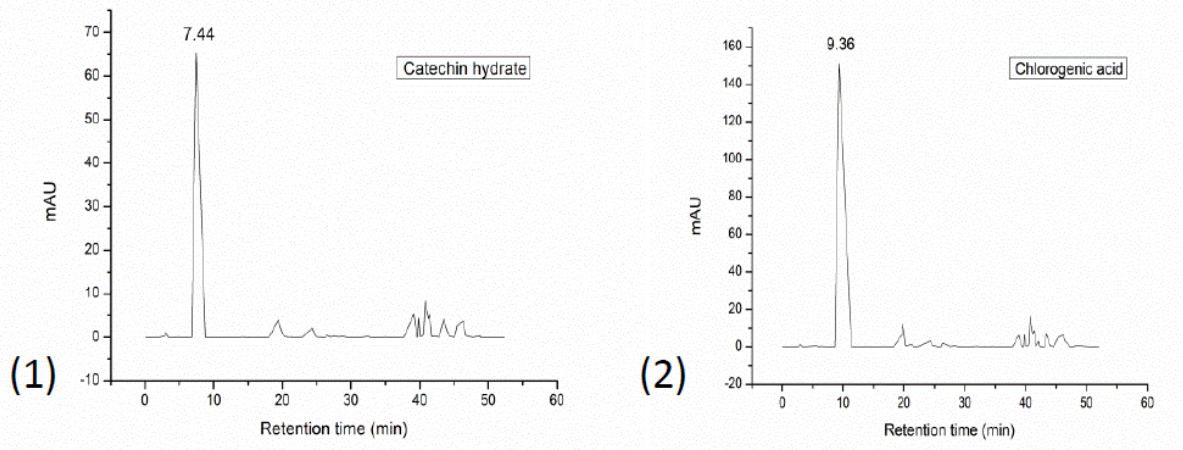
The total polyphenol content of outer and inner bracts was 7.56 and 9.44 mg phenols/g dry sample, respectively (Table 1.3 and Table 1.4). The polyphenol profiles of free and bound phenolics of outer and inner bracts were evaluated by HPLC (Fig. 1.3) and revealed the presence of 6 free phenolics (vanillic, p-coumaric, ferulic, salicylic, quercetin dihydrate, and quinic acid) in outer bracts and 8 free phenolics (catechin hydrate, chlorogenic, syringic, p-coumaric, ferulic, salicylic, quercetin dihydrate and quinic acid) in inner bracts. Moreover, both outer and inner bracts contained significant amount of bound phenolics (ferulic and salicylic acid) as illustrated in Fig. 1.3. The standard peaks are shown separately in Fig. 3. Bound phenolics were also identified and quantified in the present study and retention time (RT) for each phenolic compound is shown in Table 1.8. Higher amount of bound phenolics in outer bracts was observed as compared to inner bracts which may be referred as phenolics associated with DF. Phenolic acids (PA) occur as soluble free acids, as soluble conjugates that are esterified to sugars and other low molecular mass compounds, and as insoluble bound PAs that are mostly ester linked to cell wall polymers such as polysaccharides and lignin. The extracted PAs after alkali hydrolysis were obtained in natural forms which might be present as ester linked to cell wall polymers of banana bracts. However, based on RT of standards, the PAs in the extracts of bound phenols were identified as ferulic and salicylic acid. Several authors (Bhaskar et al., 2012; Aruna et al., 2017) have also reported that

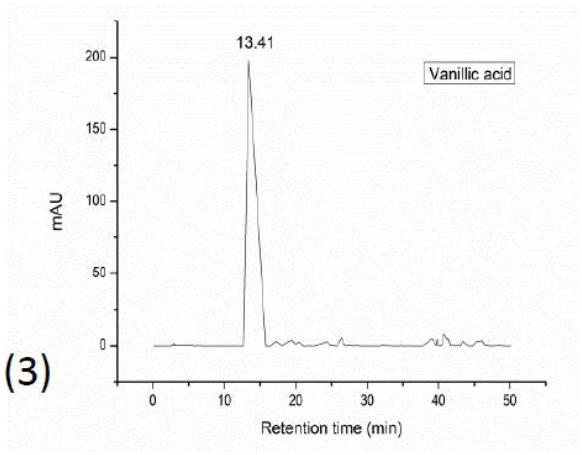
ferulic acid was obtained upon alkali hydrolysis of hemicelluloses (HA and HB) of banana (*Musa* sp var. elakki bale) flower and ferulic acid as free phenolics in *Musa paradisiaca* inflorescence. The phenolic profile by HPLC exhibited that inner and outer bracts contained substantial amount of free and bound phenolics. These phenolics provide beneficial effects on oxidative stress related disorders. The bound phenolics are relevant constituents of DF and act as carrier of dietary polyphenols to the colon and contribute to intestinal health by promoting an antioxidant environment in the colon (Macagnana et al., 2016). Moreover, these bound phenolics are mainly related with the beneficial effects of DF in the prevention and management of chronic and degenerative diseases (Aruna et al., 2017).

Table 1.8 Estimation and quantification of free and bound phenolics in outer and inner bracts of culinary banana flower

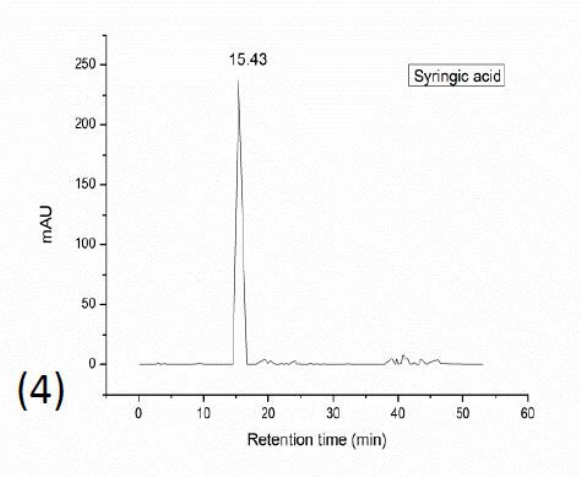
Free phenolics	Retention time (min)	Outer bract (ppm)	Inner bract (ppm)
(1)Catechin hydrate	7.44	ND	2.51±0.01
(2)Chlorogenic acid	9.36	ND	6.55±0.32
(3)Vanillic acid	13.41	1.19±0.06	ND
(4)Syringic acid	15.43	ND	1.40±0.02
(5)p-coumaric acid	19.89	34.80±0.17	6.05±0.1
(6)Ferulic acid	20.29	1.86±0.05	2.79±0.09
(7)Salicylic acid	23.58	9.86±0.47	6.23±0.21
(8)Quercetin dihydrate	25.22	5.18±0.6	1.81±0.09
(9)Quinic acid	40.77	3.63±0.05	9.17±0.11
Bound phenolics	Retention time (min)	Outer bract (ppm)	Inner bract (ppm)
(1)Ferulic acid	20.29	265.26±0.73	141.41±0.55
(2)Salicylic acid	23.58	95.75±0.86	32.92±0.14

(Mean at P<0.05 based on paired t test, values represent mean± SD; n=3); ND-not detected

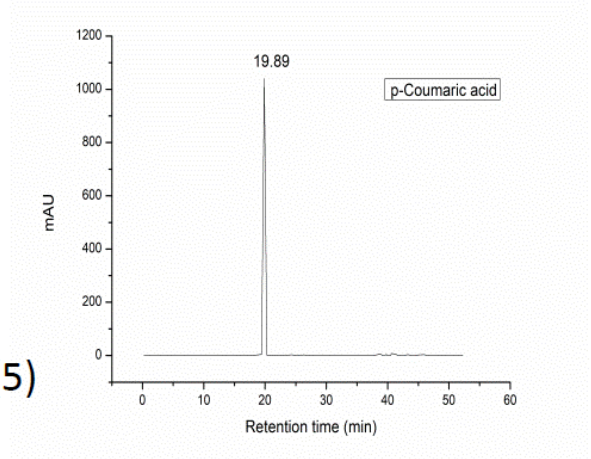



13.41
Vanillic acid
mAU
Retention time (min)

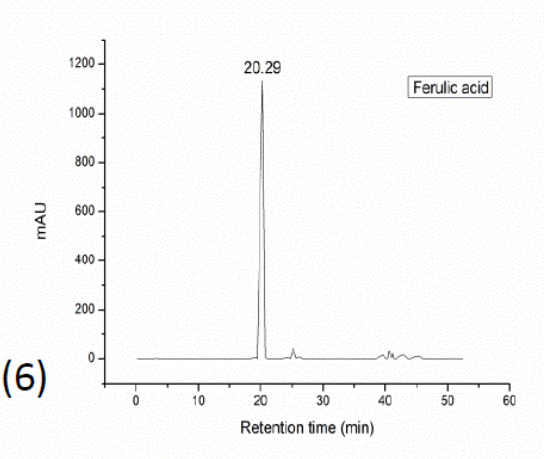
(3)


15.43
Syringic acid
mAU
Retention time (min)

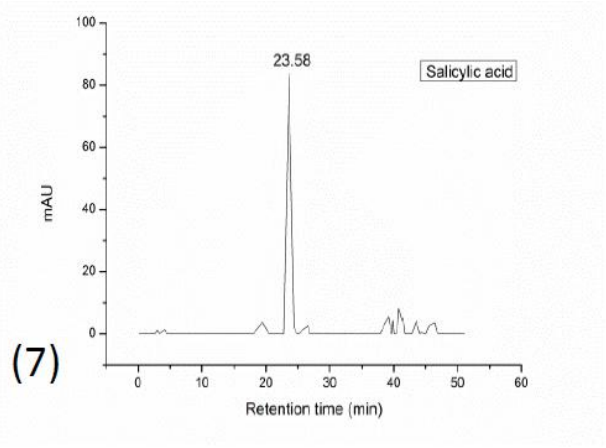
(4)


19.89
p-Coumaric acid
mAU
Retention time (min)

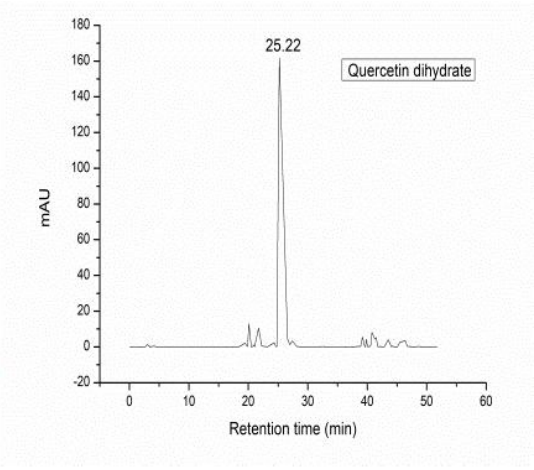
(5)


20.29
Ferulic acid
mAU
Retention time (min)

(6)


23.58
Salicylic acid
mAU
Retention time (min)

(7)


25.22
Quercetin dihydrate
mAU
Retention time (min)

(8)

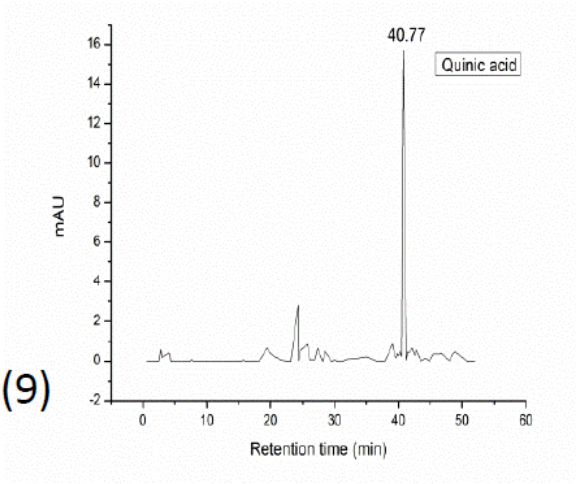


Fig.1.3 HPLC chromatogram of free and bound phenolics in outer and inner bract of culinary banana flower

The functional properties of DF-rich outer, inner bracts and cellulose (Table 1.9) revealed significantly lower bulk density and higher WHC, OHC, and WSC of outer and inner bracts as compared to cellulose. Bulk density of outer and inner bracts was observed 0.45 and 0.51 g/ml, respectively which is significantly lower than cellulose (0.55 g/ml). Bulk density is associated to the structural characteristics and particle size of a material which is related to physicochemical properties (Benitez et al., 2017). WHC of outer and inner bracts was 12.06 and 7.53 g water/ g dry sample, respectively and was significantly higher compared to cellulose (5.8 g water/ g dry sample). WHC of both outer and inner bracts was higher than that of wheat bran (5.2 g/g), corn (2.05 g/g), potato peel fibre (5.32 g/g), citrus fruits (1.65 g/g), apple (1.87 g/g), grapes (2.09 g/g) and banana (1.71 g/g) (Fernando et al., 2005). DF with high WHC avoids syneresis and improves the viscosity and texture of foods (Grigelmo-Miguel & Martina-Belloso, 1999).

Table 1.9 Functional properties of outer and inner bracts of culinary banana flower

Functional properties	Outer bract	Inner bract	Cellulose
Bulk density (g/ml)	0.45±0.02	0.51±0.01	0.55±0.01
WHC (g water/g dry sample)	12.06±0.40	7.53±0.55	5.8±0.17
OHC (g oil/g dry sample)	5.46±0.45	3.50±0.57	3.7±0.26
WSC (ml/g)	15.51±0.50	8.90±0.36	4.5±0.08

The OHC of outer and inner bracts were 5.46 and 3.5 g oil/g dry sample, respectively and was found higher than wheat bran (2.18 g/g), corn (0.87 g/g), citrus fruits (1.81 g/g), grape fruits (1.20-1.52 g/g) and apples (0.60-1.45 g/g) (Grigelmo-Miguel & Martina-Belloso, 1999). DF with improved OHC can prevent fat loss during food processing and also reduce cholesterol level in serum (Navarro-Gonzalez et al., 2011).

The WSC of outer and inner bracts was 15.51 and 8.9 ml/g, respectively and was found significantly higher when compared to cellulose (4.5 ml/g). WSC is again directly related to the soluble DF content (especially of pectin content). The WSC of outer and inner bracts revealed that it is more than several prominent vegetables viz., peas (5.26 ml/g), chickpeas (4.28 ml/g) and edible seaweed (5.7-10.5 ml/g) (Gomez-Ordonez et al., 2010). Functional properties of DF viz., WHC, OHC and WSC are important from both physiological and technological perspectives (Al-Sheraji et al., 2011). Culinary banana bracts with high WHC can be used in food system to improve density of the product, minimizing shrinkage and to improve the texture and appeal of the food products. Moreover, culinary banana bracts exhibited relatively higher OHC than several fibre rich foods, which could be useful for emulsification of some products. Additionally, due to the high hydration properties of banana bracts fibre, it can be used as anticaking and antisticking 300 agents, reduce syneresis and in stabilizing food system.

The retardation of glucose diffusion at different points of time (20, 40 and 60 min) for inner bract, outer bract, cellulose (reference) and control was taken and are illustrated in Fig. 1.4. The dialysate glucose content of control increased gradually (0.16 to 0.38 mg) with time (20 to 60 min). However, the dialysate glucose content of outer and inner bracts increased in a lower rate as compared to control and increased from 0.1 mg to 0.14 mg and 0.09 mg to 0.15 mg, respectively with time 20 to 40 min (Fig. 1.4). Hence, the glucose diffusion from dialysis tubing was potentially reduced by outer and inner bracts and was significantly ($P<0.05$) lower than cellulose. GDRI is a useful *in vitro* index which simulates the action of fibre on delaying glucose absorption in the gastrointestinal tract. It is related to several other properties of fibres, such as structural and surface properties, soluble DF content and uronic acid content (Hasnaoui et al., 2014). The soluble DF of bracts also affected the retardation of glucose diffusion. Thus, GDRI of inner and outer bracts is quite higher than cellulose which is a pure insoluble DF.

Table 1.10 Glucose dialysis retardation index (GDRI) of outer and inner bracts of culinary banana flower

Time (min)	Outer bract (%)	Inner Bract (%)	Cellulose (%)
20	40.12±0.10	41.33±0.12	22.75±0.05
40	50.79±0.17	56.67±0.17	29.50±0.03
60	62.51±0.07	59.82±0.06	30.30±0.50

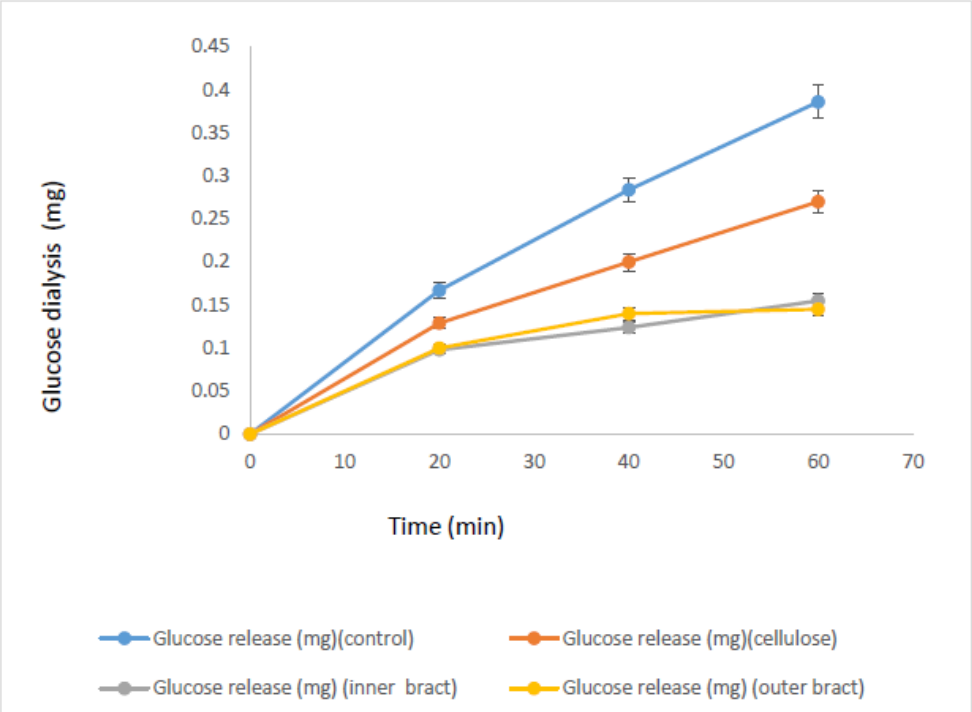


Fig.1.4 Glucose dialysis in presence of cellulose, inner and outer bracts of culinary banana flower

The GDRI of inner and outer bracts along with cellulose are presented in Table 10. The GDRI of outer and inner bracts at 60 min was 62.51 and 59.82%, respectively and these values are strikingly higher compared to several fibre-rich byproducts, such as artichoke fibre (27%), mango peel (21%) and asparagus fibre (18-47%) as reported by Hasnaoui et al. (2014). The plausible reasons might be attributed to the antihyperglycemic effects of banana flower. Banana flower (*Musa* sp. var. elakki bale) was found to have antidiabetic properties and inhibit the formation of AGEs (Advanced Glycation End Products) in streptozotocin induced diabetic rats (Bhaskar et al., 2011). Moreover, the results suggested that different parts of culinary banana bracts have potential in controlling postprandial blood glucose level and could be used in antidiabetic remedies formulations. This could also be used as low calorie and fibre-rich ingredients for dietetic snacks.

Objective 2: Optimization of soluble and insoluble fiber extraction from culinary banana flower (*Musa* ABB) based on RSM

Materials and methods

2.1. Extraction of dietary fibre from culinary banana flower (*Musa* ABB)

Extraction of dietary fibre from culinary banana flower (*Musa* ABB) was done by the method as given by Bouaziz et al. (2014) with some modifications. In this method, hot water was used to extract DF from banana flower powder in a jar and homogenized and maintained in a thermostatic bath. After

solubilisation of free sugar and SDF, IDF were recovered by centrifugation. Its concentration was determined by water rinsing and centrifugation until the residue was free of sugar. SDF are settled over-night in ethanol and stirred and then recovered by centrifugation. Its concentration was also determined by rinsing with ethanol. The residues obtained was dried in a hot-air oven and then ground in a laboratory grain mill and passed through 0.5 mm mesh to give the dietary fibre concentrates and stored in an air tight container for subsequent analysis (Bouaziz et al., 2014).

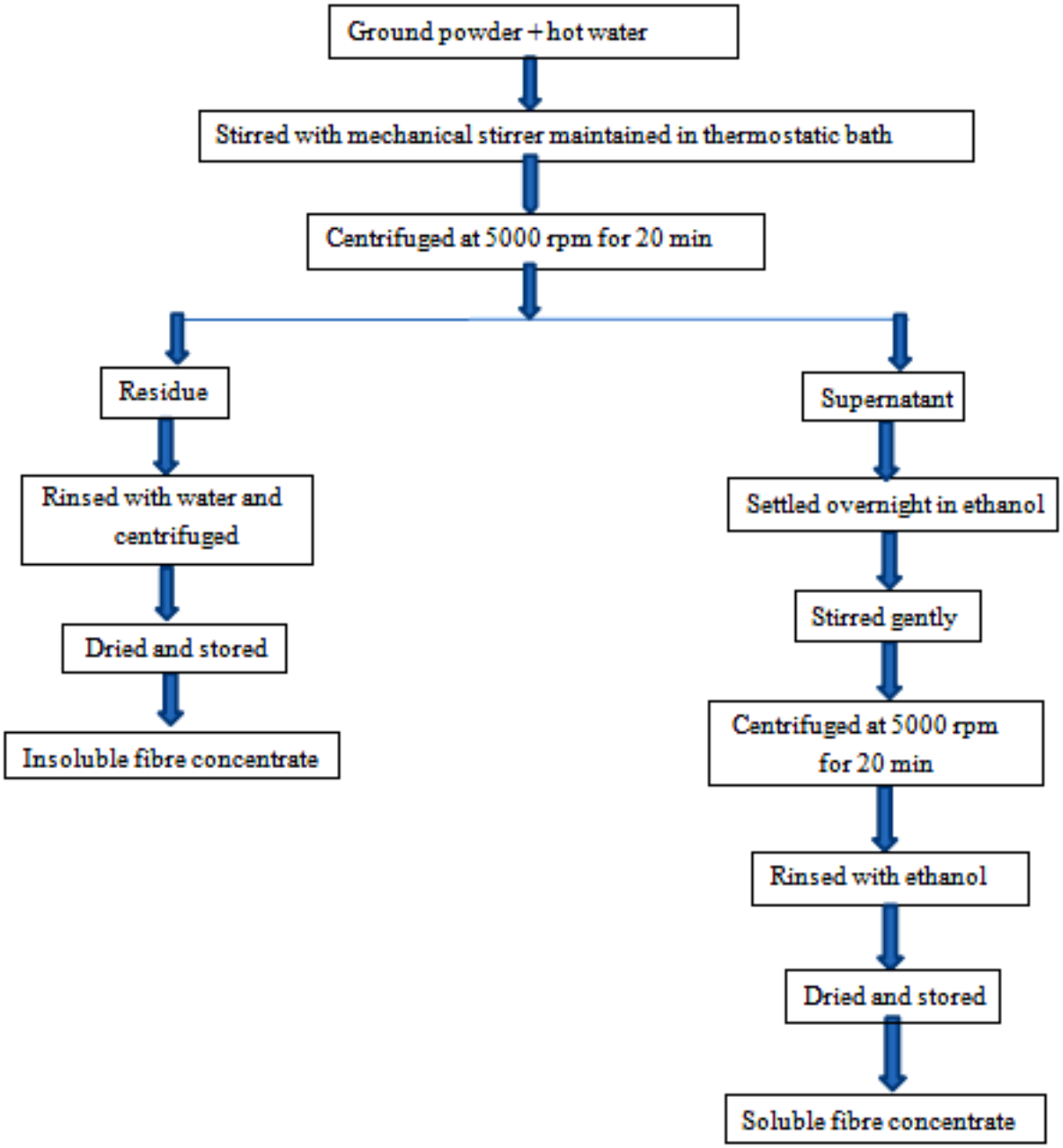


Fig.2.1 Dietary fibre extraction process from culinary banana flower (Bouaziz et al., 2014)

2.2. Characterization of the dietary fiber concentrates from culinary banana flower (*Musa* ABB)

2.2.1. Physicochemical properties of dietary fibre

2.2.1.1. Water Holding Capacity

Water holding capacity was determined by adding 25 ml of distilled water to 1 g of dry sample. Then it was stirred and incubated at room temperature for 1 h. After that tubes were centrifuged at 3000 RPM for 20 min. The supernatant was decanted and tubes were allowed to drain for 10 min. The residue was weighed and water holding capacity was calculated as gram of water per gram of dry sample (Rodriguez-Ambriz et al., 2008).

2.2.1.2. Oil Holding Capacity

Oil holding capacity was determined by adding 25 ml of olive oil to 1 g of dry sample. Then it was stirred and incubated at room temperature for 1 h. After that tubes were centrifuged at 3000 RPM for 20 min. The supernatant was decanted and tubes were allowed to drain for 10 min. The residue was weighed and oil holding capacity was calculated as gram of oil per gram of dry sample (Rodriguez-Ambriz et al., 2008).

2.2.1.3. Solubility and Swelling Test

The determination of swelling power and solubility is according to the method as proposed by Leach et al. (1959) with some modifications. In a preweighed centrifuge tube, 1 g of dietary fibre was placed. Then, 20 ml of distilled water was added and stirred at room temperature for 30 min and then centrifuged at 5000 RPM for 15 min. The supernatant obtained was poured into a preweighed petriplate and placed in the oven at 105℃ to evaporate. After that the solid residue in the petriplate was weighed again. The difference in the weight was calculated as percentage solubility.

$$\%\ \text{solubility} = \left(\frac{\text{Weight of the dried sediment}}{\text{weight of the original sample}}\right) \times 100\%$$

The residue in the tube was then weighed and the swelling power was determined by the following equation:

$$\%\ \text{swelling power} = \left(\frac{\text{weight of the residue in the tube}}{\text{weight of the original sample}}\right) \times (100\ -\ \%\ \text{solubility}) \times 100$$

2.2.1.4. pH

pH was determined by making powder suspension [8%(w/v)], which was stirred for 5 min and then allowed to stand for 30 min, filtered and the pH of filtrate was measured (Suntharalingam & Ravindran, 1993).

2.2.1.5. Determination of DPPH radical scavenging activity

Antioxidant activity (AOA) was measured using the method described by Brand et al. (1995). Antioxidant activity was calculated as percent discoloration.

$$\text{Radical scavenging activity (\%)} = \left(\frac{1 - \text{As}}{\text{Ac}}\right) \times 100\%$$

Where, As = absorbance of DPPH and extract

Ac = absorbance of the control

2.2.1.6. Bulk density

For determination of bulk density, 2 g of powdered sample was placed in a 50 ml graduated cylinder and packed by tapping the cylinder by a rubber sheet until a constant volume was obtained. The bulk density of the sample was expressed as g of sample per ml (Bouaziz et al., 2014).

2.2.1.7. Morphological studies by Scanning Electron Microscope (SEM) analysis:

The structural and morphological features of the extracted dietary fibre was analysed by SEM. It determines the particle structure of both soluble and insoluble dietary fibre powders attached to a double sided adhesive tape on metal stud, coated with silver under vacuum and was examined at 20 kV and magnification of 1000X (scale bar 10 micro meter) (Bouaziz et al., 2014).

2.2.1.8. X-ray powder diffraction (XRD) Measurements:

XRD measurements were carried out to investigate crystallinity and phase composition of the extracted dietary fibre. XRD was performed at 30 kV and 15 mA. The measurement was carried out at a 2θ range at 15-75 ºC in room temperature. The degree of crystallinity was calculated from the peak area under the curve using the following equation (Qu et al., 2017).

$$\text{Dc (\%)} = \left(\frac{\text{Ac} \times 100}{\text{Ac} + \text{AA}}\right)$$

Where, Dc =degree of crystallinity

Ac = crystallized area 26

AA= amorphous area on the X- ray diffractogram.

2.2.1.9. Differential Scanning Calorimetry (DSC)

Measurements were performed on a calorimeter DSC 220C connected to a disc station 5200H (Seiko, J-Tokyo). Approximately 15 mg of the dispersion are accurately weighed in an aluminium crucible and fused on cold. The probes were measured against an empty reference crucible in a temperature range of 20-200℃ at a heating rate of 10℃/min (Zhang et al., 2017).

Results and discussion

2.3. Modelling of the extraction process of soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) from culinary banana (*Musa* ABB) flower

The total number of experimental runs was generated as per response surface methodology design of Box-Behnken design. The total number of runs was 62 having seven independent variables and two dependent variables or responses. The condition of responses was shown in Table 2.1.

Table 2.1 Experimental design of the two dependent factors with seven independent variables using Box-Behnken Design

Run	P:W	Temp. (℃)	NaCl (%)	RPM	Alcohol (times)	Time (min)	Amplitude	SDF (g/100 g sample)	IDF (g/100g sample)
1	0.05	75	1.50	300	0.25	150	75	0.02735	0.5247
2	0.03	75	1.50	450	1.13	30	90	0.0367	0.4554
3	0.07	75	2.00	450	0.25	90	75	0.04167	0.5627
4	0.07	75	2.00	450	2.00	90	75	0.06724	0.5126
5	0.05	75	1.50	450	1.13	90	75	0.04506	0.4664
6	0.05	75	1.50	300	0.25	30	75	0.0371	0.5097
7	0.05	90	1.00	450	1.13	150	75	0.04197	0.4814
8	0.05	60	1.00	450	1.13	150	75	0.0639	0.5323
9	0.05	90	1.50	450	2.00	90	60	0.07061	0.5304
10	0.05	60	2.00	450	1.13	30	75	0.037	0.4357
11	0.05	75	1.50	450	1.13	90	75	0.04506	0.4864
12	0.07	75	1.50	450	1.13	30	60	0.04987	0.3557
13	0.05	90	1.00	450	1.13	30	75	0.0357	0.4487
14	0.03	75	2.00	450	2.00	90	75	0.06934	0.6016
15	0.05	75	2	600	1.13	90	60	0.05691	0.5164
16	0.07	60	1.5	300	1.13	90	75	0.06076	0.4958

17	0.05	60	1.5	450	2	90	90	0.0704	0.5011
18	0.05	75	1.5	450	1.13	90	75	0.04506	0.4864
19	0.05	75	1.5	600	2	30	75	0.0392	0.4406
20	0.05	75	1.5	600	0.25	30	75	0.03146	0.4568
21	0.05	75	2	600	1.13	90	90	0.0584	0.5025
22	0.05	75	1.5	450	1.13	90	75	0.04506	0.4864
23	0.05	75	1.5	450	1.13	90	75	0.05426	0.5442
24	0.03	75	2	450	0.25	90	75	0.0368	0.5898
25	0.05	75	1.5	600	2	150	75	0.081629	0.5825
26	0.03	60	1.5	300	1.13	90	75	0.05428	0.54784
27	0.03	75	1.5	450	1.13	150	60	0.0483	0.5695
28	0.07	90	1.5	600	1.13	90	75	0.06852	0.5068
29	0.07	60	1.5	600	1.13	90	75	0.05865	0.5014
30	0.05	60	1	450	1.13	30	75	0.0332	0.3605
31	0.03	90	1.5	300	1.13	90	75	0.05511	0.5849
32	0.05	75	2	300	1.13	90	60	0.05485	0.5095
33	0.05	90	2	450	1.13	30	75	0.0361	0.5573
34	0.05	75	1.5	450	1.13	90	75	0.04506	0.5264
35	0.05	75	1.5	600	0.25	150	75	0.02929	0.5895
36	0.05	75	1.5	300	2	150	75	0.08002	0.5017
37	0.03	75	1	450	0.25	90	75	0.0325	0.5698
38	0.03	75	1.5	450	1.13	30	60	0.05826	0.4711
39	0.05	75	1	300	1.13	90	90	0.06582	0.4202
40	0.07	75	1.5	450	1.13	30	90	0.05286	0.4761
41	0.07	75	1.5	450	1.13	150	90	0.0739	0.4946
42	0.05	60	1.5	450	0.25	90	60	0.02712	0.5251
43	0.05	90	1.5	450	0.25	90	60	0.02401	0.5421
44	0.03	75	1	450	2	90	75	0.06123	0.5901
45	0.03	75	1.5	450	1.13	150	90	0.04802	0.4762
46	0.07	75	1.5	450	1.13	150	60	0.05087	0.47878
47	0.05	75	1	300	1.13	90	60	0.05485	0.4862
48	0.05	60	1.5	450	2	90	60	0.05085	0.5479
49	0.07	75	1	450	2	90	75	0.06489	0.4987
50	0.05	90	1.5	450	0.25	90	90	0.02634	0.5721
51	0.05	75	1	600	1.13	90	60	0.0639	0.4889
52	0.05	60	1.5	450	0.25	90	90	0.02966	0.5223

53	0.03	90	1.5	600	1.13	90	75	0.05644	0.59593
54	0.07	75	1	450	0.25	90	75	0.02884	0.5034
55	0.07	90	1.5	300	1.13	90	75	0.06921	0.5057
56	0.05	75	2	300	1.13	90	90	0.05421	0.5215
57	0.05	90	2	450	1.13	150	75	0.06857	0.5147
58	0.03	60	1.5	600	1.13	90	75	0.05582	0.564
59	0.05	60	2	450	1.13	150	75	0.06852	0.5256
60	0.05	90	1.5	450	2	90	90	0.0685	0.52233
61	0.05	75	1	600	1.13	90	90	0.0459	0.4795
62	0.05	75	1.5	300	2	30	75	0.0385	0.4987

2.3.1. Statistical analysis and model fitting

RSM was applied to study the effect of powder-water ratio, temperature, NaCl, RPM of mixing, alcohol, time, amplitude on the dependent variables i.e. soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) for extraction process. Table 2.2 represents the statistical data representing the analysis of variance (ANOVA) of the test. The sequential sum of squares, F values and corresponding coefficient of determination (adj. R^2) were also shown. Variance and regression analysis were carried out to fit the suggested quadratic model and investigated the statistical significance of model factors. The adequacy of the model was investigated by the p-values and F-values of the regression model. It was observed that the predicted models for all the response variables were adequately fitted to the observed experimental data ($p< 0.0001$). In Table 2.2, the linear, square and interaction effects of each response variables were also presented. The lack-of-fit value for each response was observed and there was no lack-of-fit ($p< 0.0001$) in any response model. The R^2 value for SDF was found out to be 0.9081 and for IDF to be 0.9455. The difference between R^2 and R^2 values adjusted for SDF was 0.1237 and for IDF was 0.0733. Thus, these results showed that the models can establish optimum condition for extraction of SDF and IDF.

2.3.2. Effect of the process variables on response (SDF)

The values of the coefficient for the SDF were used for a final predictive equation.

Final Equation in Terms of Coded Factors

SDF yield (Y_1) = 0.0465933 + 0.00310333A + 0.000455B + 0.00237125C - ±0.000247542D + 0.0162612E + 0.00818287F + 0.00084625G + 0.00210875AB + 0.00034625AC + -0.00070875AD + 4.375e-05AE + 0.002585AF + 0.0059825AG + 0.0023225BC + 0.00015125BD + 0.00303625BE + -0.002935BF - 0.00273375BG + 0.00214CD - 0.00083375CE + 0.0033775CF + 0.000985CG + 0.000751125DE + 0.00106112DF - 0.003355DG + 0.0119836EF + 0.00157125EG + 0.005165FG + 0.00589224A_2 + 0.00158974B_2 + 0.00228162 C_2 + 0.00577343D_2 - 0.00445344E_2 - 0.00234469F_2 + 0.00220662G_2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

SDF extraction increases significantly when powder-water ratio was 0.03 followed by a decline with increase in powder-water ratio as shown by a three-dimensional surface plot. Fig. 2.2 represents the effect of varying powder-water ratio and alcohol times on the yield of SDF. It is obvious that with increase in alcohol times, there was increase in the total yield of SDF. Fig. 2.3 represents the effect of time and temperature on yield SDF which showed that the yield of SDF increased with the increase in time and temperature during mixing. Fig.2.2 shows that the decrease in value of powder-water ratio and increase in the value of amplitude during sonication process resulted in more yield of SDF. More the alcohol used during separation process and more the time of heating or mixing, more was the yield of SDF, which was shown in Fig. 2.4. Increase in the time of heating or mixing and increase in the amplitude of ultrasonication increased the yield of SDF as shown in Fig. 2.4. A quadratic relationship was observed between SDF yield and powder-water ratio.

Table 2.2 ANOVA for Response Surface Quadratic Model for Soluble Dietary Fibre

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0121	35	0.0003	7.34	< 0.0001	Significant
A-PW	0.0002	1	0.0002	4.89	0.036	
B-TEMP	4.97E-06	1	4.97E-06	0.1052	0.7483	
C-NACL	0.0001	1	0.0001	2.86	0.103	
D-RPM	1.47E-06	1	1.47E-06	0.0311	0.8613	
E-ALCOHOL	0.0063	1	0.0063	134.33	< 0.0001	
F-TIME	0.0016	1	0.0016	34.02	< 0.0001	
G-AMPLITUDE	0	1	0	0.3638	0.5516	
AB	0	1	0	0.753	0.3935	
AC	9.59E-07	1	9.59E-07	0.0203	0.8878	
AD	4.02E-06	1	4.02E-06	0.0851	0.7729	
AE	1.53E-08	1	1.53E-08	0.0003	0.9858	
AF	0.0001	1	0.0001	1.13	0.2972	
AG	0.0003	1	0.0003	6.06	0.0208	
BC	0	1	0	0.9134	0.348	
BD	1.83E-07	1	1.83E-07	0.0039	0.9508	
BE	0.0001	1	0.0001	1.56	0.2226	
BF	0.0001	1	0.0001	1.46	0.238	
BG	0.0001	1	0.0001	1.27	0.2709	

CD	0	1	0	0.7755	0.3866	
CE	5.56E-06	1	5.56E-06	0.1177	0.7343	
CF	0.0001	1	0.0001	1.93	0.1764	
CG	7.76E-06	1	7.76E-06	0.1643	0.6885	
DE	4.51E-06	1	4.51E-06	0.0955	0.7597	
DF	9.01E-06	1	9.01E-06	0.1907	0.666	
DG	0.0001	1	0.0001	1.91	0.1792	
EF	0.0011	1	0.0011	24.32	< 0.0001	
EG	0	1	0	0.4181	0.5236	
FG	0.0002	1	0.0002	4.52	0.0432	
A^2	0.0005	1	0.0005	9.92	0.0041	
B^2	0	1	0	0.7222	0.4032	
C^2	0.0001	1	0.0001	1.49	0.2335	
D^2	0.0004	1	0.0004	9.53	0.0048	
E^2	0.0003	1	0.0003	5.67	0.0249	
F^2	0.0001	1	0.0001	1.57	0.2212	
G^2	0.0001	1	0.0001	1.39	0.2488	
Residual	0.0012	26	0			
Lack of Fit	0.0012	21	0.0001	3.91	0.068	Not significant
Pure Error	0.0001	5	0			
Cor Total	0.0134	61				

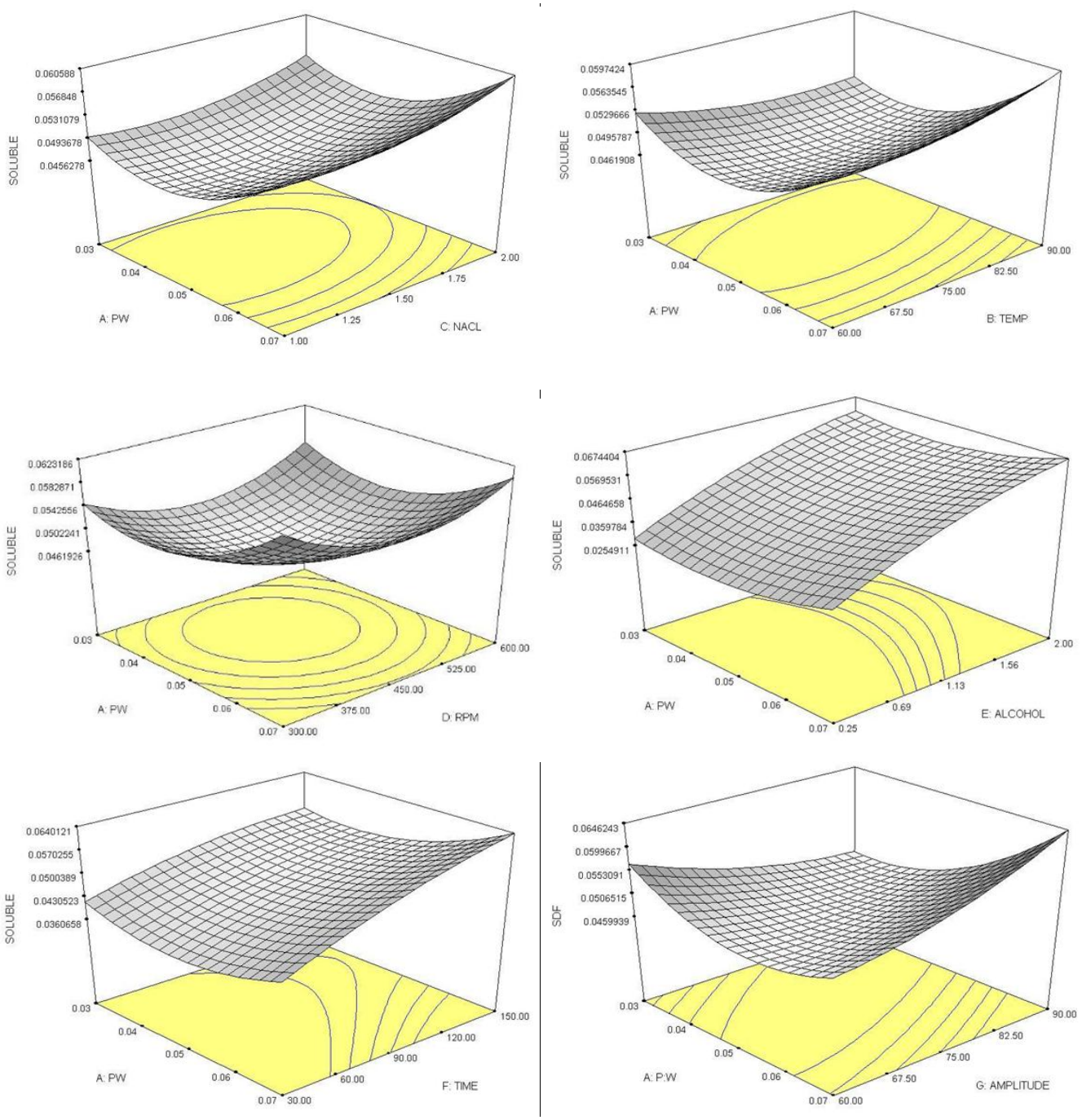


Fig.2.2 Effect of different process variables on yield of SDF: (a) powder-water ratio and temperature, (b) powder-water ratio and NaCl, (c) powder-water ratio and RPM, (d) powder-water ratio and alcohol times, (e) powder-water ratio and time, (f) powder-water ratio and amplitude

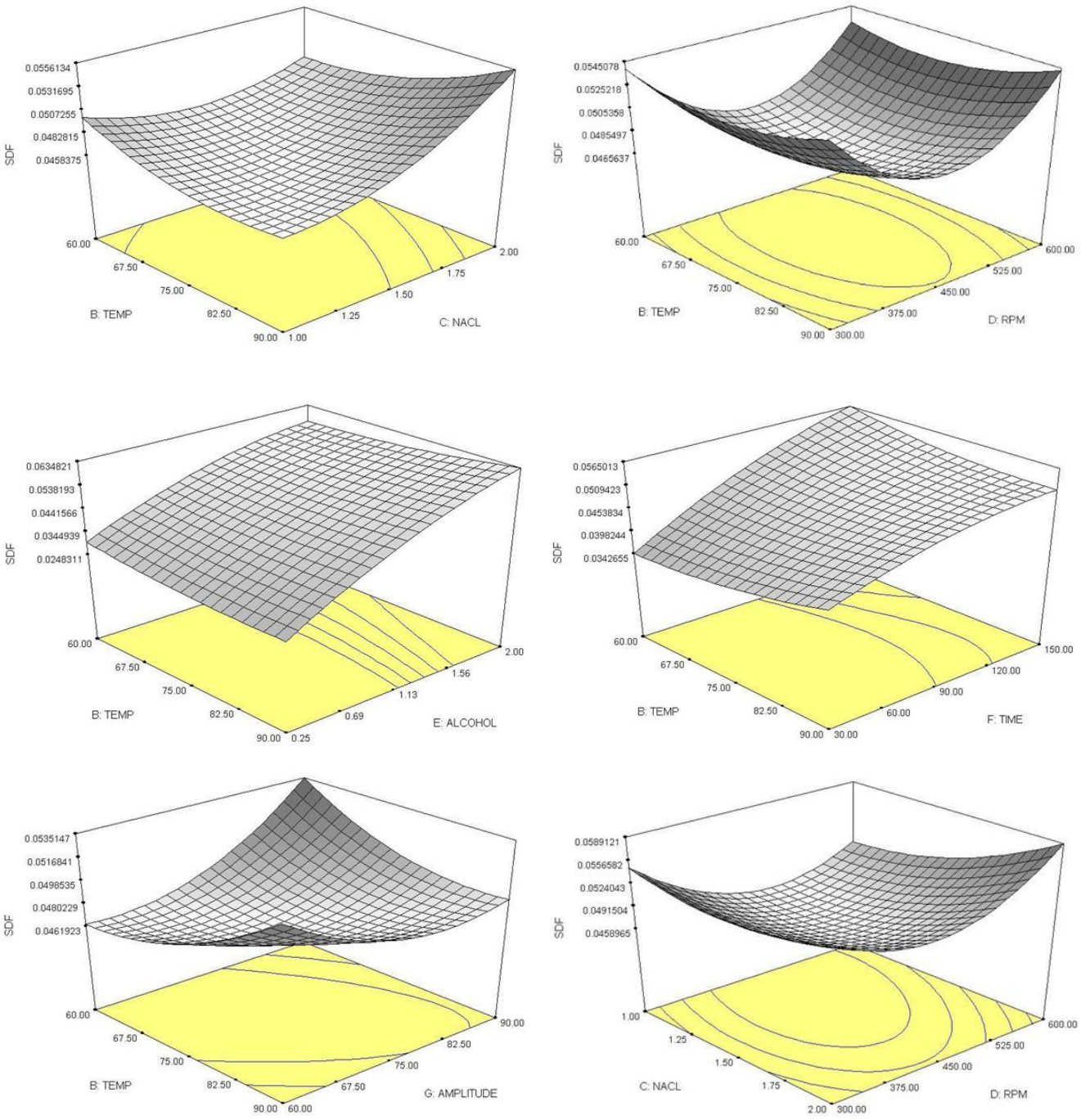


Fig.2.3 Effect of different process variables on yield of SDF: (a) temperature and NaCl, (b) temperature and RPM, (c) temperature and alcohol times, (d) temperature and time of heating, (e) temperature and time of amplitude of sonication, (f) NaCl and RPM of mixing

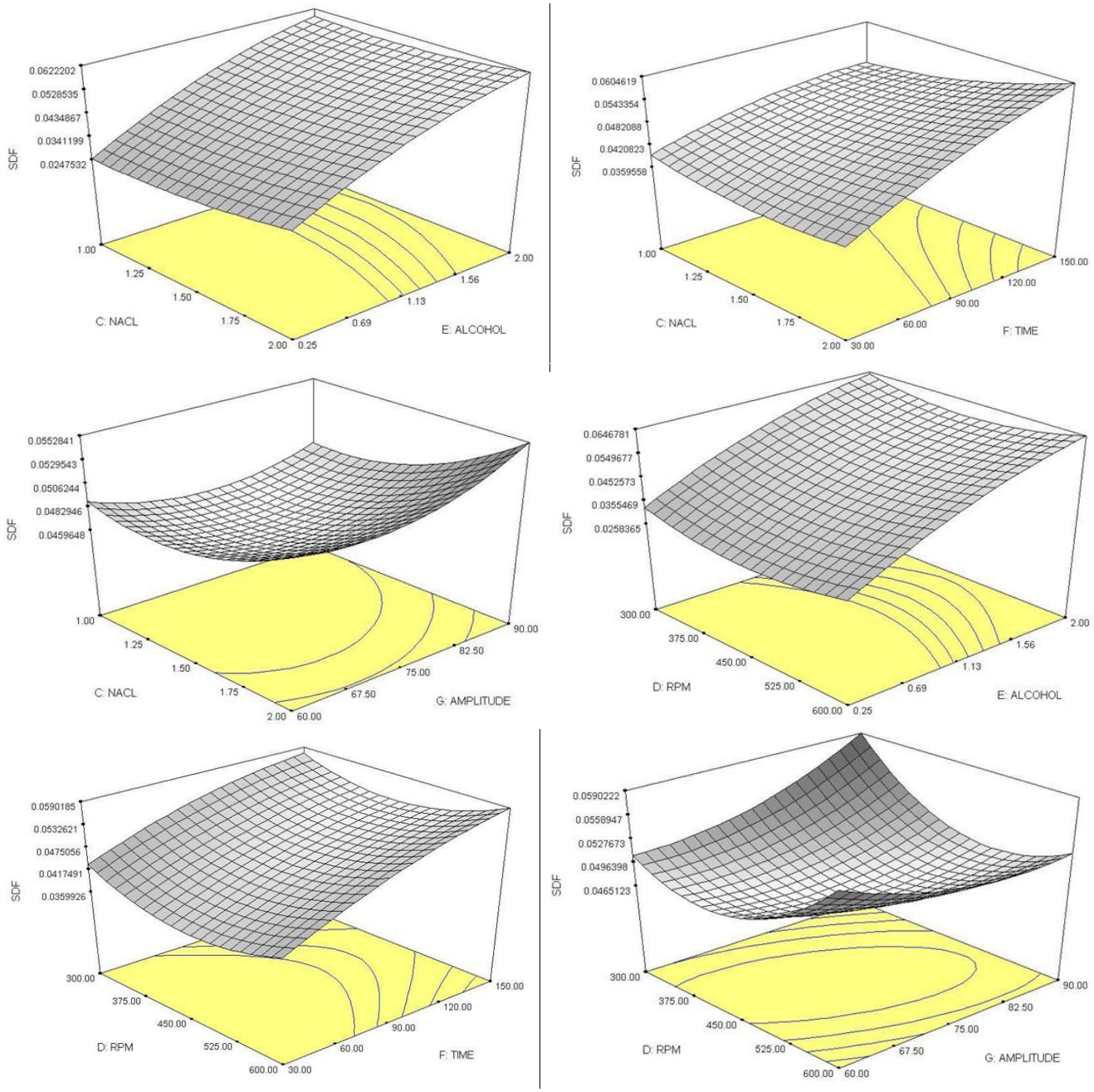


Fig.2.4 Effect of different process variables on yield of SDF: (a) NaCl and alcohol times, (b) NaCl and time of extraction, (c) NaCl and time of amplitude of ultrasonication, (d) RPM of mixing and alcohol time, (e) RPM of mixing and time of mixing, (f) RPM of mixing and amplitude of ultrasonication

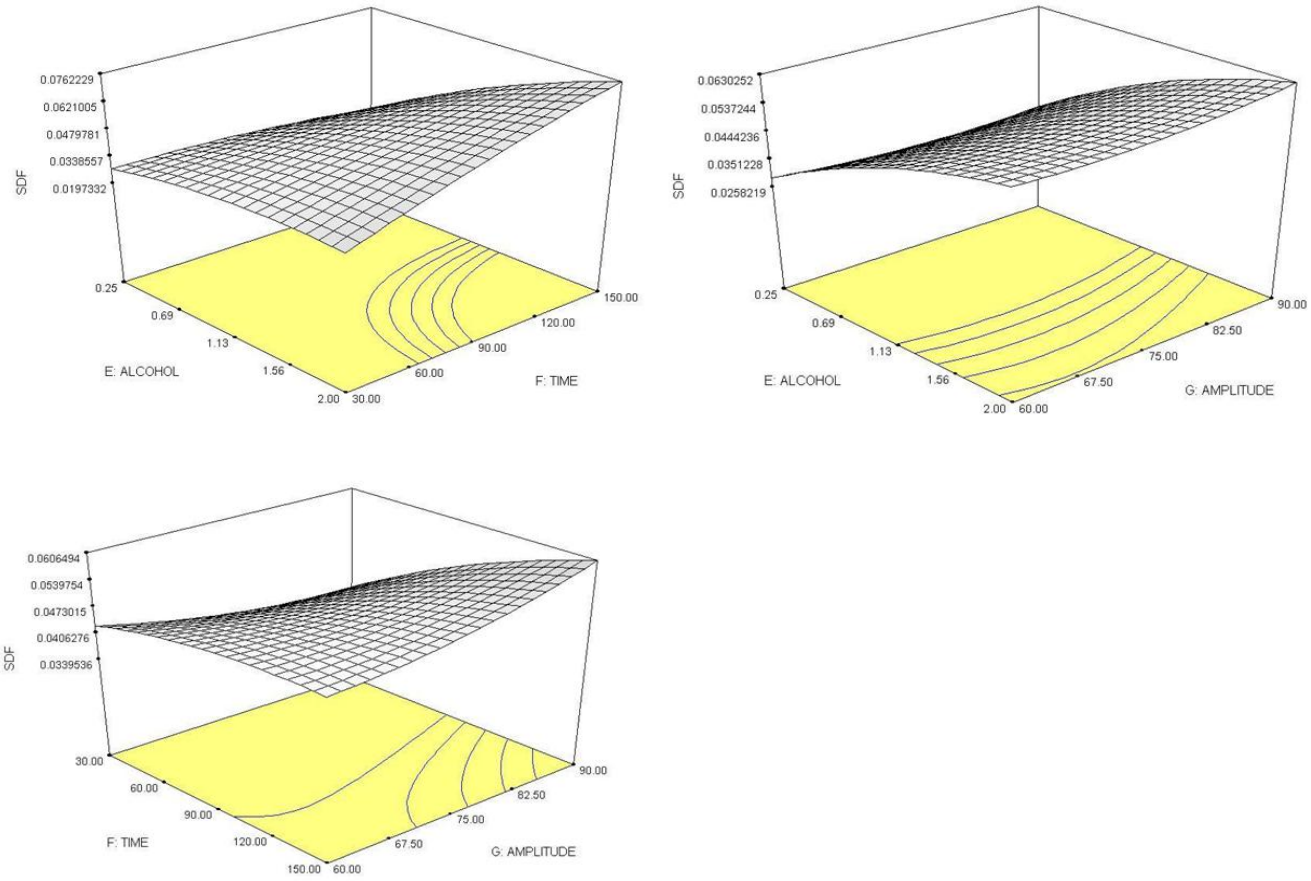


Fig.2.5 Effect of different process variables on yield of SDF: (a) alcohol times and time of mixing, (b) alcohol times and amplitude of ultrasonication, (c) time of mixing and amplitude of ultrasonication

2.3.3. Effect of the process variables on response (IDF)

The value of the coefficient for the IDF was used as a final predictive equation:

IDF Yield (Y_2) = 0.499367 - 0.0301621A + 0.0126175 B + 0.020425 C + 0.00493292 D - 0.00582375E + 0.0335492F -0.00323958G - 0.00671125AB + 0.0052125AC -0.00256125AD - 0.0108625AE + 0.0027975AF + 0.0306525AG + 0.009175BC - 0.00120375BD - 0.00788375BE - 0.03395BF + 0.00894125BG -0.0092625CD - 0.0067375CE - 0.01965CF + 0.0091875CG + 0.00135DE + 0.032075DF + 0.0038375DG - 0.00035EF - 0.010258 EG - 0.0227725FG + 0.0183042A_2+ 0.0128949B_2 - 0.000373889C_2 + 0.00723049D_2+ 0.0362905E_2 - 0.0298626F - 0.0156358G_2

The optimal level of time and temperature for obtaining the maximum insoluble dietary fibre is shown by a three-dimensional surface plot (Fig. 2.6) which shows the effect of IDF and the independent variables according to above equation. Fig. 2.5 represents the effect of varying temperature and powder-water ratio, with increase in temperature, the yield increases and decreases with the increase of powder-water ratio. Fig. 2.5 also represents the effect of NaCl and powder-water ratio, which shows yield increases with increase of NaCl amount. Fig. 2.5 represents the effect of powder-water ratio and RPM, which shows yield increases with increase of RPM of mixing. Increase

in alcohol volume beyond the volume of solution increases the yield of IDF. It has also been shown that as the time of mixing increases, yield of IDF increases.

Table 2.3 ANOVA for Response Surface Quadratic Model for Insoluble Dietary Fibre

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1497	35	0.0043	12.89	< 0.0001	significant
A-PW	0.0218	1	0.0218	65.8	< 0.0001	
B-TEMP	0.0038	1	0.0038	11.52	0.0022	
C-NACL	0.01	1	0.01	30.18	< 0.0001	
D-RPM	0.0006	1	0.0006	1.76	0.1961	
E-ALCOHOL	0.0008	1	0.0008	2.45	0.1294	
F-TIME	0.027	1	0.027	81.41	< 0.0001	
G-AMPLITUDE	0.0003	1	0.0003	0.759	0.3916	
AB	0.0004	1	0.0004	1.09	0.307	
AC	0.0002	1	0.0002	0.655	0.4256	
AD	0.0001	1	0.0001	0.158	0.6941	
AE	0.0009	1	0.0009	2.84	0.1036	
AF	0.0001	1	0.0001	0.189	0.6676	
AG	0.0075	1	0.0075	22.65	< 0.0001	
BC	0.0007	1	0.0007	2.03	0.1661	
BD	0	1	0	0.035	0.8532	
BE	0.0005	1	0.0005	1.5	0.2319	
BF	0.0092	1	0.0092	27.79	< 0.0001	
BG	0.0006	1	0.0006	1.93	0.1768	
CD	0.0007	1	0.0007	2.07	0.1623	
CE	0.0004	1	0.0004	1.09	0.3051	
CF	0.0031	1	0.0031	9.31	0.0052	
CG	0.0007	1	0.0007	2.04	0.1656	
DE	0	1	0	0.044	0.8356	
DF	0.0082	1	0.0082	24.81	< 0.0001	
DG	0.0001	1	0.0001	0.355	0.5564	
EF	9.80E-07	1	9.80E-07	0.003	0.9571	
EG	0.0008	1	0.0008	2.54	0.1233	
FG	0.0041	1	0.0041	12.5	0.0015	
A^2	0.0045	1	0.0045	13.63	0.001	
B^2	0.0022	1	0.0022	6.77	0.0151	
C^2	1.89E-06	1	1.89E-06	0.006	0.9405	
D^2	0.0007	1	0.0007	2.13	0.1567	
E^2	0.0178	1	0.0178	53.58	< 0.0001	
F^2	0.012	1	0.012	36.28	< 0.0001	
G^2	0.0033	1	0.0033	9.95	0.004	
Residual	0.0086	26	0.0003			
Lack of Fit	0.0043	21	0.0002	0.236	0.9919	not significant
Pure Error	0.0043	5	0.0009			
Cor Total	0.1584	61				

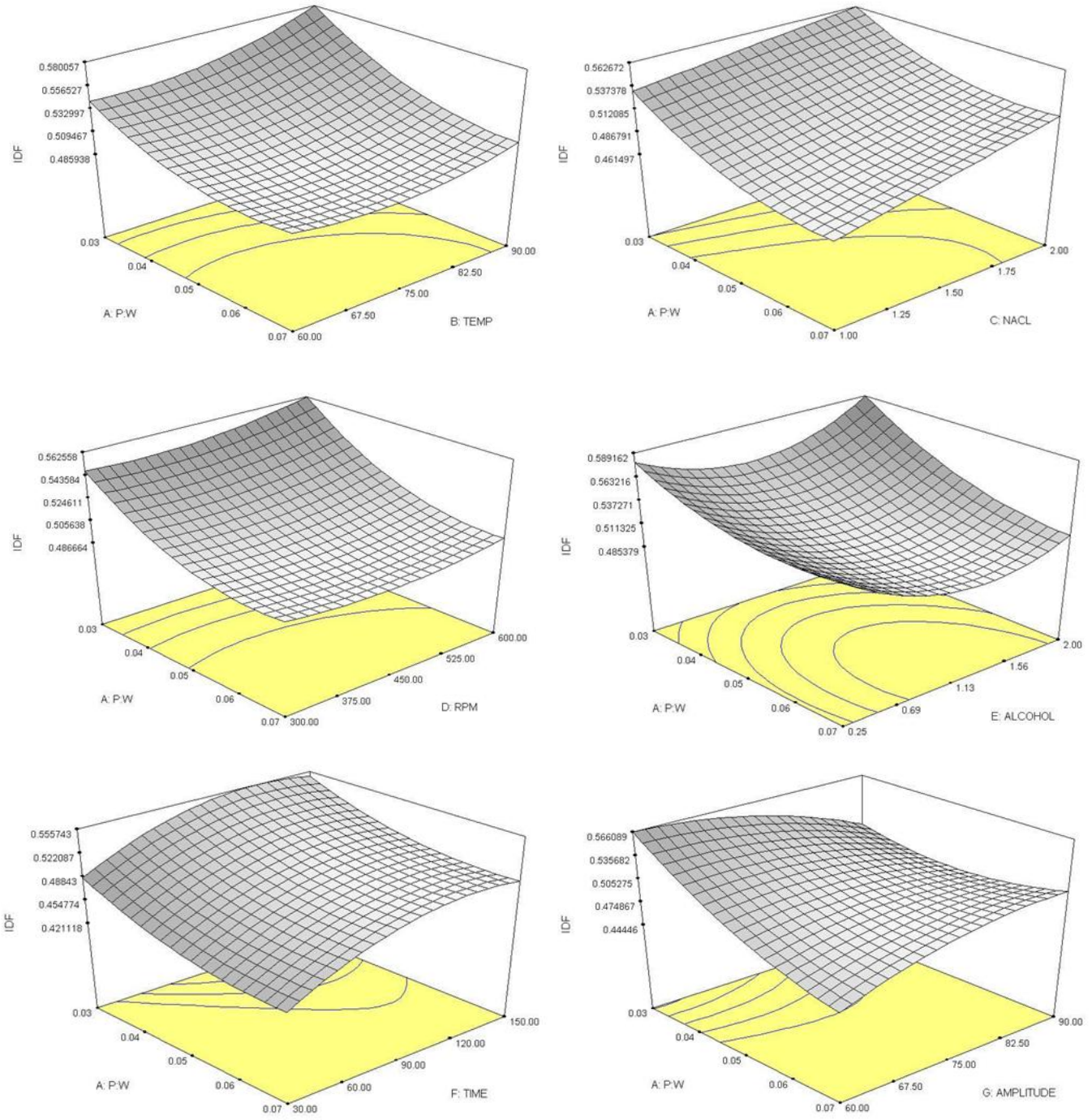


Fig.2.6 Effect of different process variables on yield of IDF: (a) powder-water ratio and temperature, (b) powder-water ratio and NaCl, (c) powder-water ratio and RPM, (d) powder-water ratio and alcohol times, (e) powder-water ratio and time, (f) powder-water ratio and amplitude

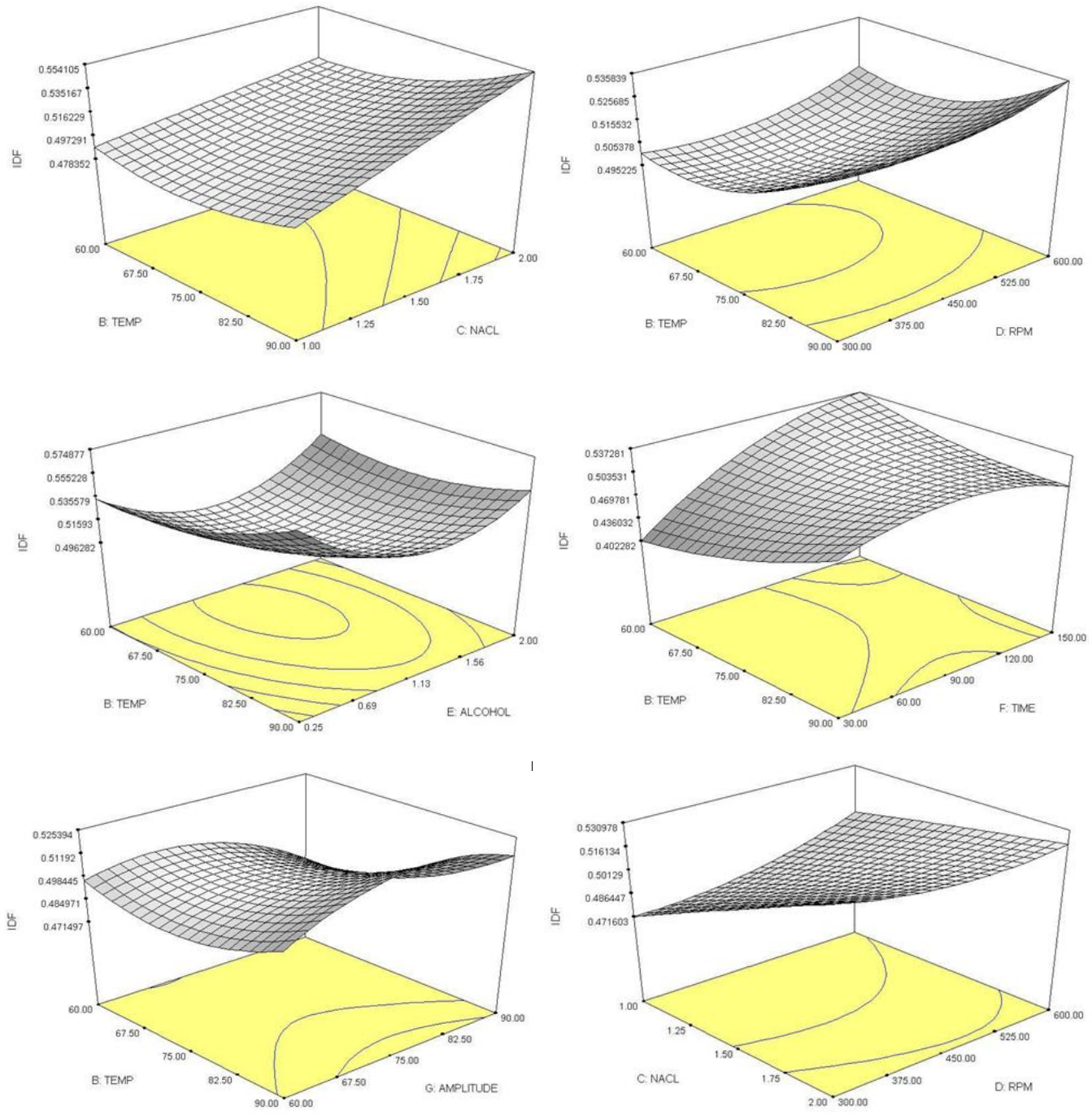


Fig.2.7 Effect of different process variables on yield of IDF: (a) temperature and NaCl, (b) temperature and RPM, (c) temperature and alcohol times, (d) temperature and time of heating, (e) temperature and time of amplitude of sonication, (f) NaCl and RPM of mixing

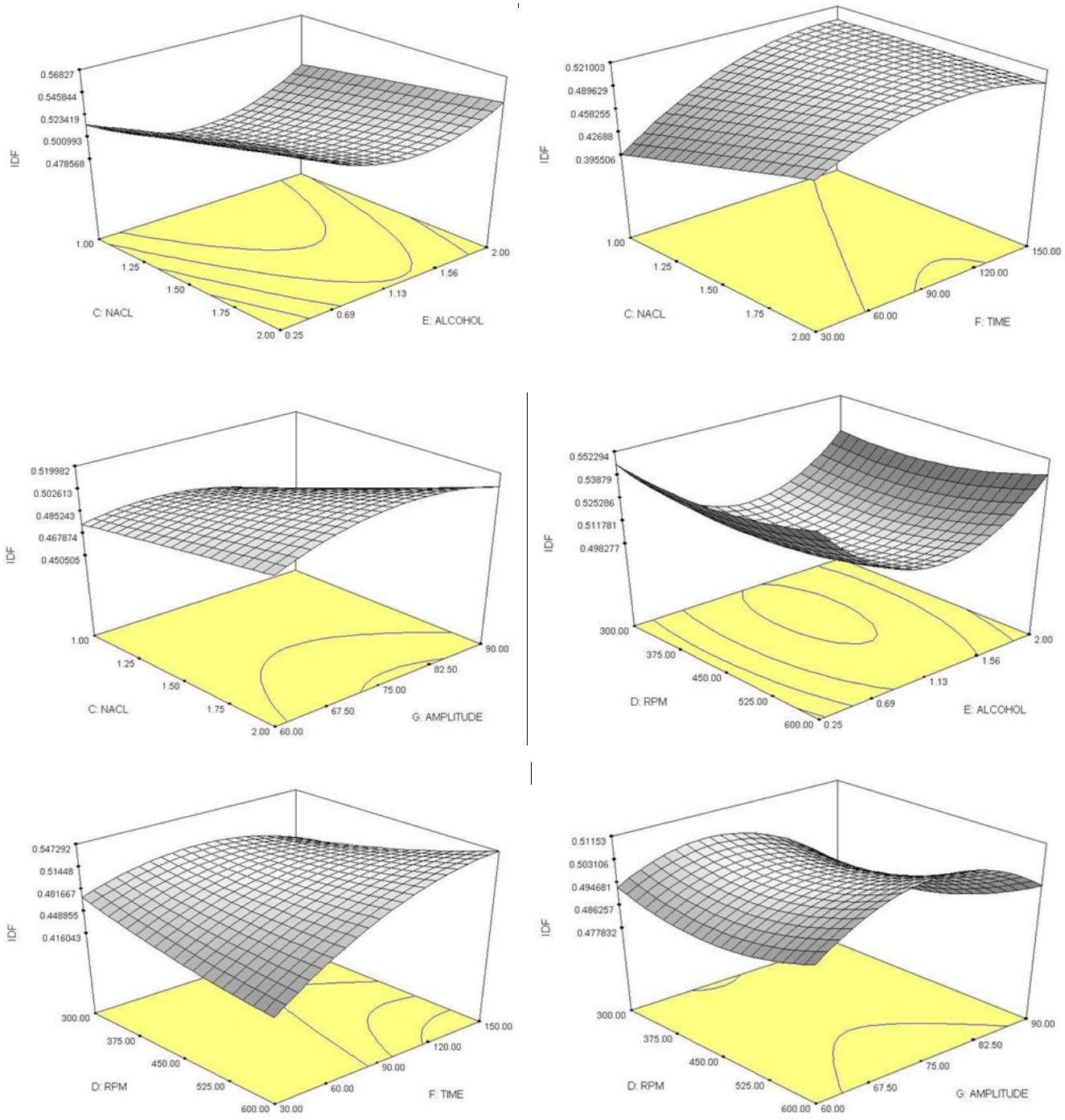


Fig.2.8 Effect of different process variables on yield of IDF: (a) NaCl and alcohol times, (b) NaCl and time of extraction, (c) NaCl and time of amplitude of ultrasonication, (d) RPM of mixing and alcohol time, (e) RPM of mixing and time of mixing, (f) RPM of mixing and amplitude of ultrasonication

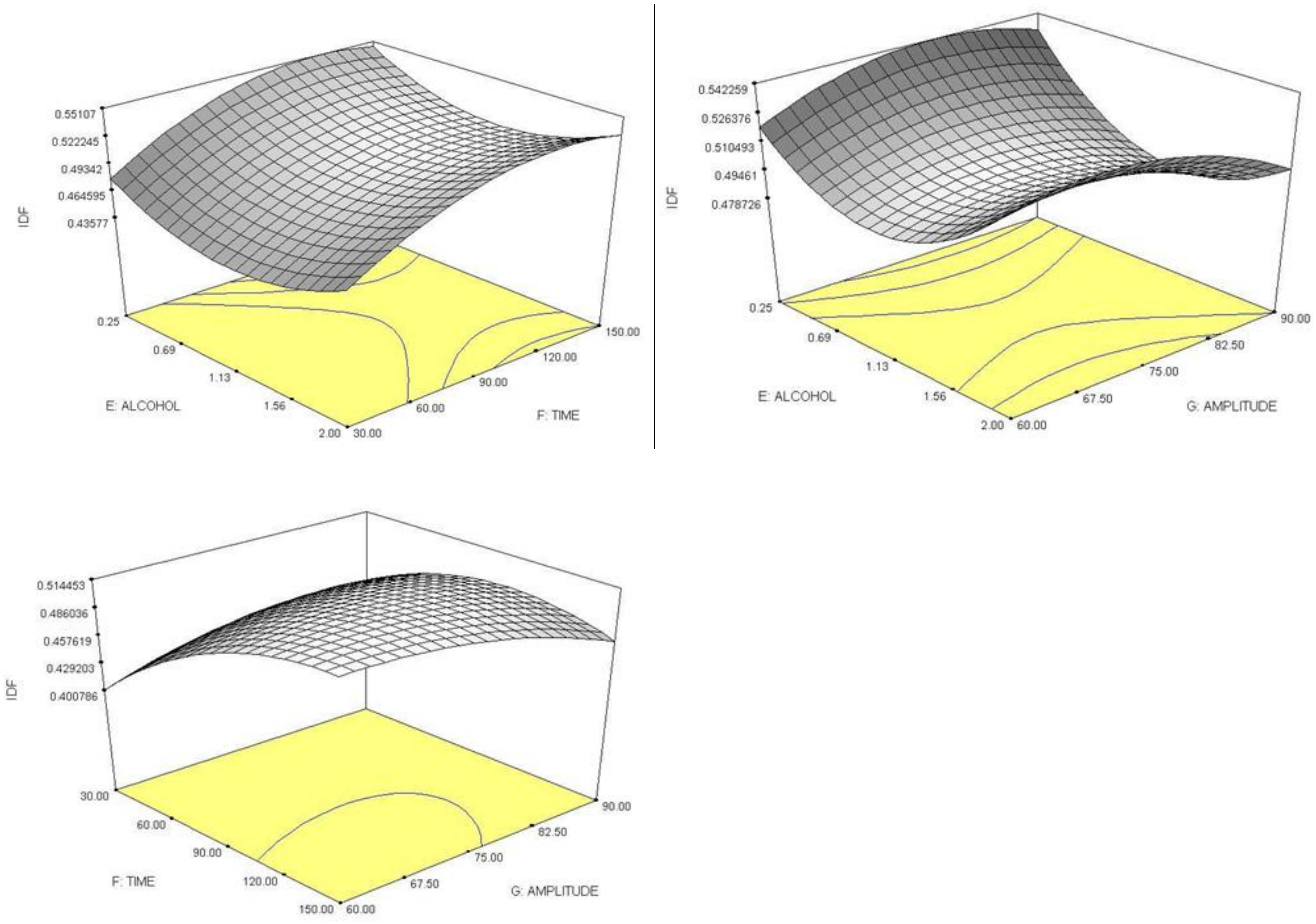


Fig.2.9 Effect of different process variables on yield of IDF: (a) alcohol times and time of mixing, (b) alcohol times and amplitude of ultrasonication, (c) time of mixing and amplitude of ultrasonication

Fig.2.10 Soluble dietary fibre of culinary banana flower (*Musa* ABB)

Fig.2.11 Insoluble dietary fibre of culinary banana flower (*Musa* ABB)

Physicochemical properties of extracted dietary fibre

Table 2.4 Results of physicochemical properties of SDF and IDF

Sample	WHC (g water/g dry sample)	OHC (g oil/g dry sample)	Solubility (%)	Swelling	pH	Bulk density
SDF	6.28	0.98	7.2	7.01	5.69	5.28
IDF	4.10	1.68	6.41	7.52	5.98	6.17

DPPH radical scavenging activity

Dietary fibre is a major source of antioxidant property. The percentages of scavenging activity of soluble, insoluble and total dietary fibre are 4.68, 32.10 and 26.41 respectively.

Table 2.5 Percent scavenging of SDF, IDF and total DF

Dietary fibre concentrate	% scavenging activity
Soluble dietary fibre	4.68
Insoluble dietary fibre	32.10
Total dietary fibre	26.41

X-ray powder diffraction (XRD) patterns of soluble and insoluble dietary fibre

Fig. 2.12 and 2.13 showed the XRD pattern of soluble and insoluble dietary fibre. As shown in the Fig. 2.12, soluble dietary fibre has sharp peak at 40 to 50℃ and insoluble dietary fibre has sharp peak at 21 to 23℃ which indicates the presence of A type crystalline diffraction pattern.

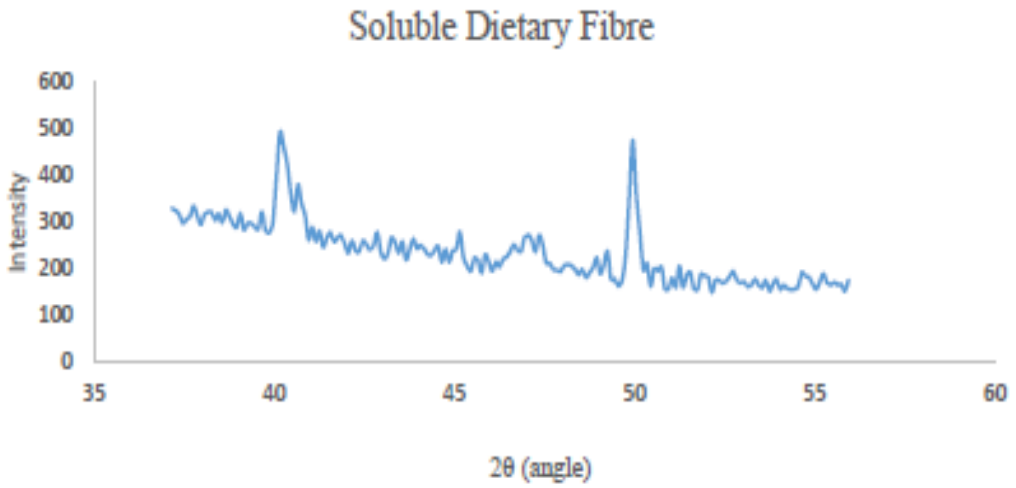


Fig.2.12. X-ray powder diffraction (XRD) pattern of soluble dietary fibre (SDF)

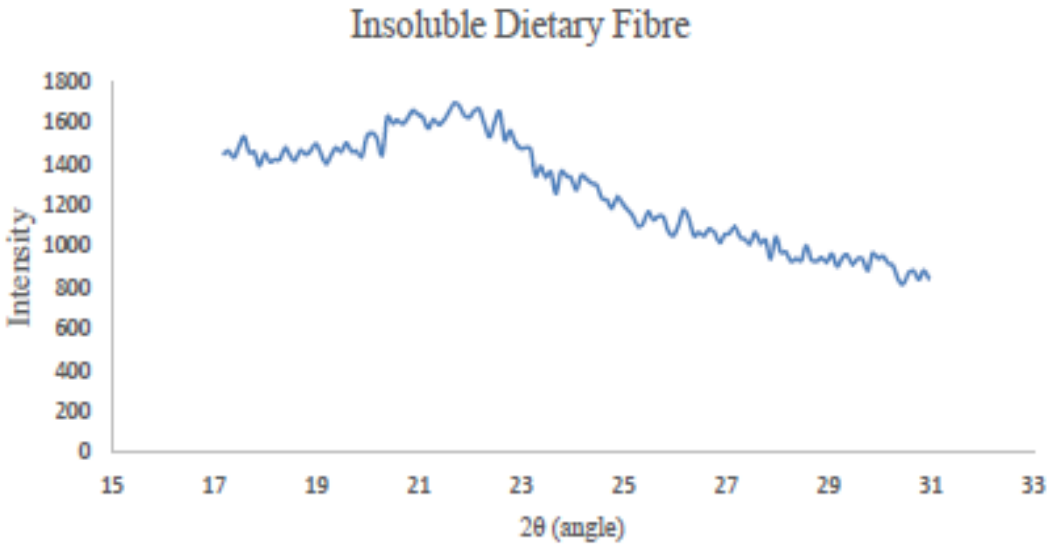


Fig.2.13 X-ray powder diffraction (XRD) pattern of insoluble dietary fibre (IDF)

Particle size and microstructure

Fig. 2.14 and 2.15 shows the SEM micrograph of extracted SDF and IDF. SEM analysis shows that the surface structure of soluble fibre particle are amorphous in structure and the insoluble fibres are more fibrous structure and stronger because it absorbs less water and forms compact and stronger bonds of fibre.

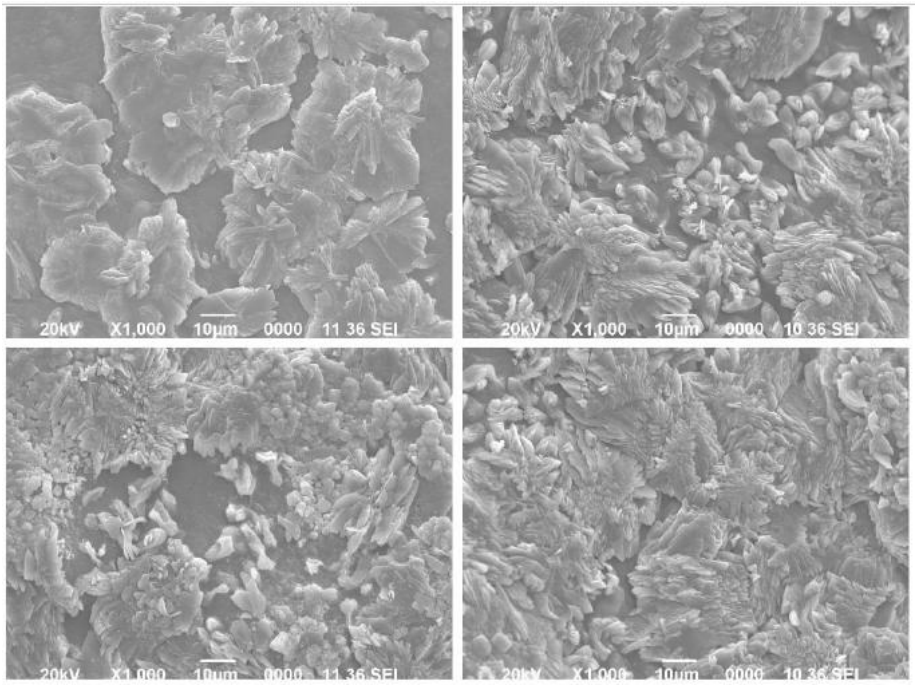


Fig.2.14 SEM micrograph of soluble dietary fibre SDF (1000X magnification)

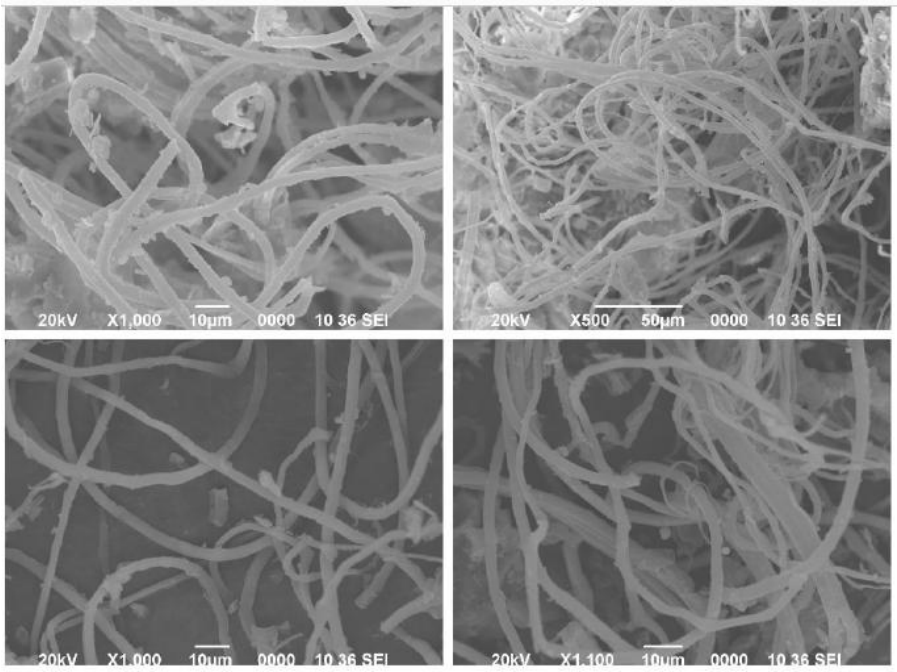


Fig.2.15 SEM micrograph of insoluble dietary fibre IDF (500X, 1000X and 1100X magnification)

Differential scanning calorimetry of fibre

The thermal properties of the extracted dietary fibre were analysed using Differential Scanning Calorimtery, DSC thermograph showed an endothermic behaviour of both SDF and IDF. For SDF, DSC was calculated at a temperature range of 20 to 200℃. The onset temperature was 81.1℃, peak temperature was 118.4 ℃ and the end temperature was 144.5 ℃. For IDF, DSC was calculated at a temperature range of 20 to 200℃. The onset temperature was 106.0 ℃, peak temperature was 122.8 ℃ and the end temperature was 148.9 ℃. Fig. 2.16 and 2.17 showed the DSC of SDF and IDF.

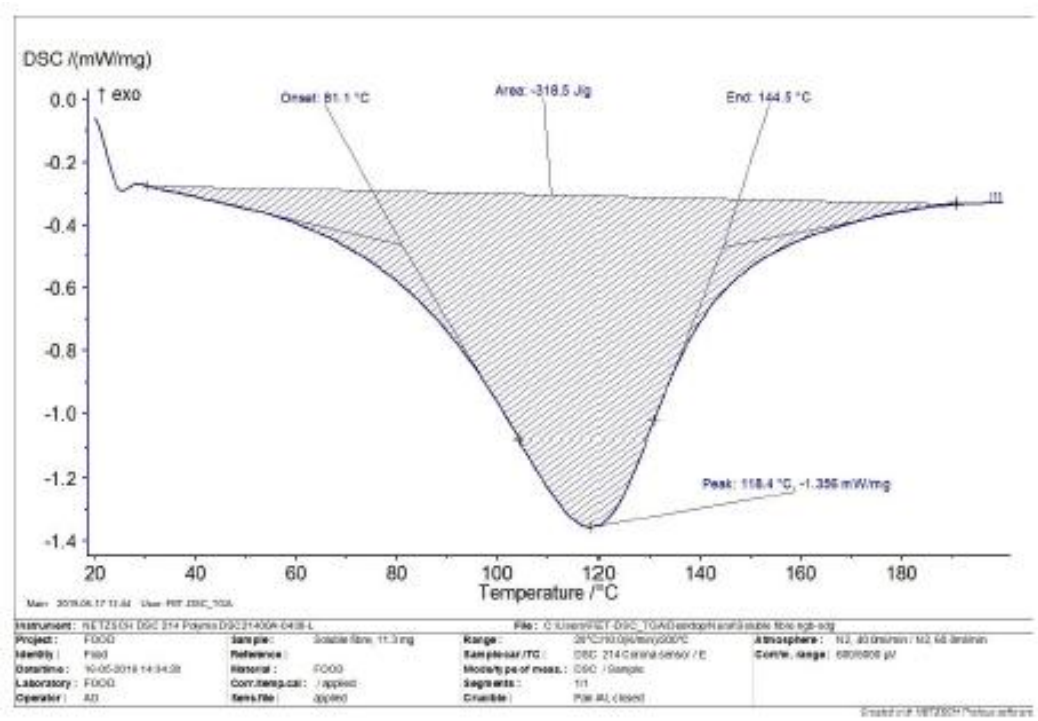


Fig.2.16 Differential scanning calorimetry (DSC) of soluble dietary fibre (SDF)

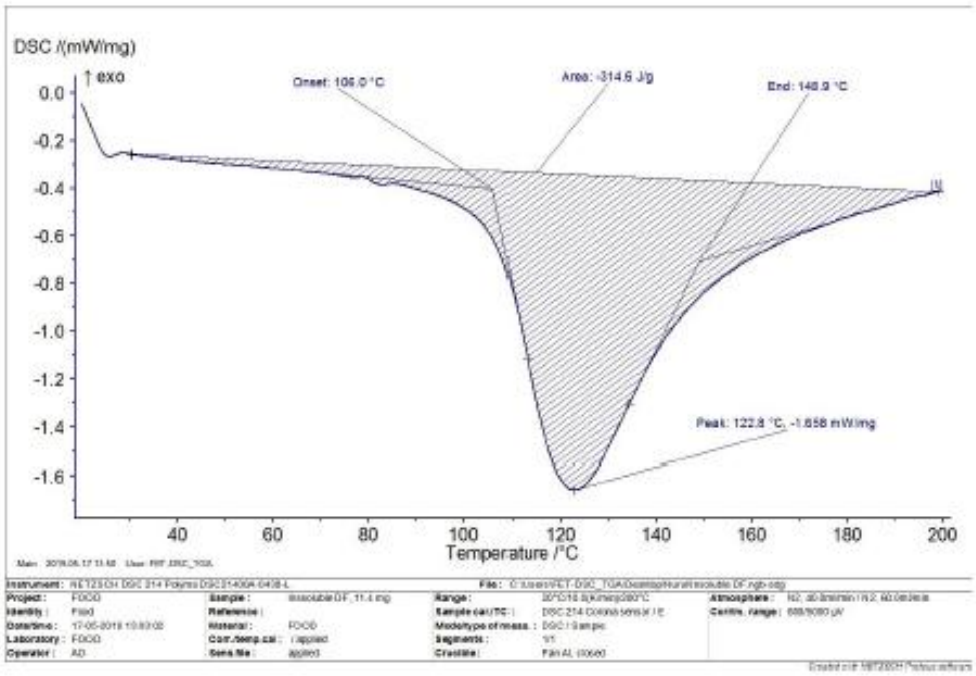


Fig.2.17 Differential scanning calorimetry (DSC) of insoluble dietary fibre (IDF)

Objective 3: Toxicity studies of dietary fibre

Materials and methods

Blood samples required for the toxicity study were collected from Health Centre, Tezpur University. The studies were carried out following the methods given below:

3.1. Membrane stability test of dietary fibre extract on RBC cells

Human blood, 4% sodium citrate buffer (1:5)

⇩

Centrifuged (2442 rpm, 5 min, room temperature)

⇩

Pellets taken and washed with PBS (pH 7.4) buffer three times

⇩

Pellets resuspended into PBS (1:20)

⇩

Taken 160µL of the above sample in each tube

⇩

-ve control (PBS)

⇩

+ve control (Triton X)

⇩

Sample added (25, 50,100µg/mL) (40µL)

⇩

Incubated at 37ºC, Water bath, 35 min

⇩

Kept in ice for 5 min

⇩

10 min agitation

⇩

Centrifuged for 5 min (6500rpm)

⇩

Supernatant placed on 96 well plate (20 µL each)

⇩

180 µL PBS

⇩

Reading at 535 nm

Fig.3.1 Flowchart for membrane stability test of dietary fibre extract on RBC cells

3.2. Haemolysis Activity of dietary fibre extract on RBC cells

Human blood, 4% sodium citrate buffer (1:5)

⇩

Centrifuged (2442 rpm, 5 min, room temperature)

⇩

Pellets taken and washed with PBS (pH 7.4) buffer three times

⇩

Pellets resuspended into PBS (1:20)

⇩

Taken 160µL of the above sample in each tube

⇩

-ve control (PBS)

⇩

+ve control (DW)

⇩

Sample added (25, 50,100µg/mL) (40µL)

⇩

Incubated at 37ºC, Water bath, 35 min

⇩

Kept in ice for 5 min

⇩

10 min agitation

⇩

Centrifuged for 5 min (6500rpm)

⇩

Supernatant placed on 96 well plate (20 µL each)

⇩

180 µL PBS

⇩

Reading at 535 nm

Fig.3.2 Flowchart for haemolysis activity of dietary fibre extract on RBC cells

3.3. HPBMC (here we take only the neutrophils, eosinophils and basophils of WBC)

Human blood, 4% sodium citrate buffer (1:5)

⇩

Histopaq buffer (Differential centrifugation) (1:1)

⇩

Centrifuged (2442 rpm, 5 min, room temperature)

⇩

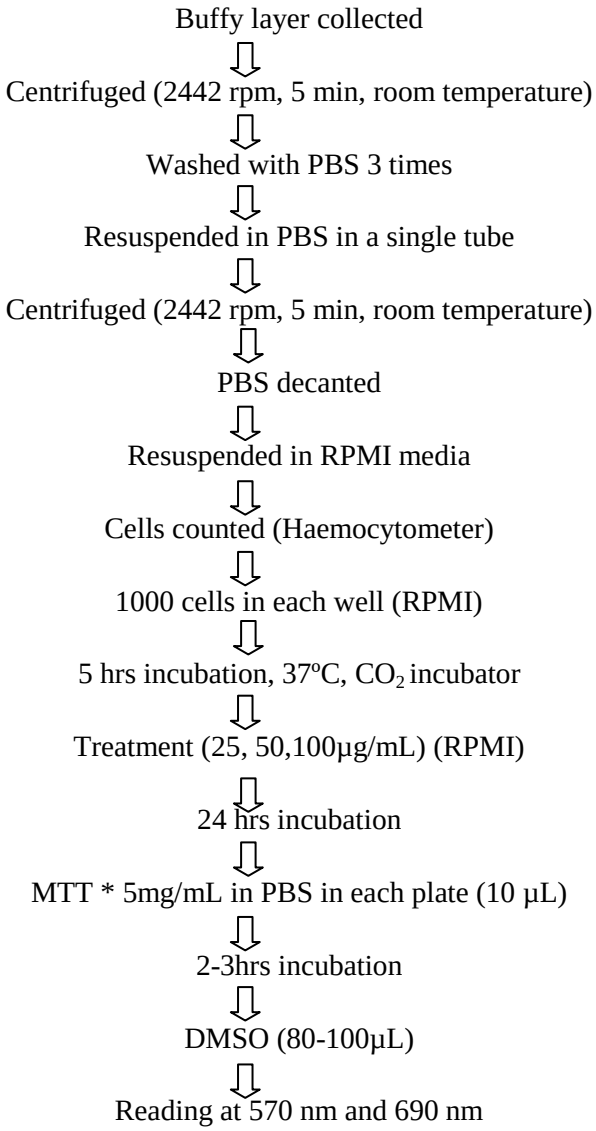


Fig.3.3 Flowchart for HPBMC (here we take only the neutrophils, eosinophils and basophils of WBC)

3.4. MTT Assay of dietary fibre extract on HT-29 cells

Media discarded from cell cultures. For adherent cells, media should be carefully aspirated. For suspension cells, 96 well plate spinned at 2988 rpm, 4°C for 5 minutes and carefully aspirated the media

⇩

50 µL of serum-free media and 50 µL of MTT solution added into each well

⇩

Plate incubated at 37°C for 3 hours

⇩

After incubation, 150 µL of MTT solvent added into each well

⇩

Plate wrapped in foil and shaked on an orbital shaker for 15 minutes

⇩

Occasionally, pipetting of the liquid may be required to fully dissolve the MTT formazan

⇩

Absorbance read at OD=590 nm

Fig.25. Flowchart for MTT Assay of dietary fibre extract on HT-29 cells and Caco-2 cells

Results and discussion

3.5. Membrane stability test of dietary fibre on RBC cells

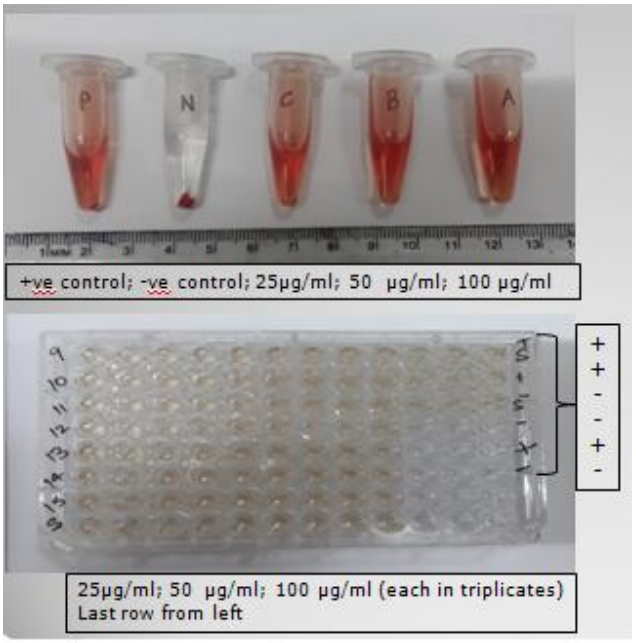


Fig.3.4 Membrane stability test of dietary fibre on RBC cells

Table 3.1 Membrane stability of dietary fibre on RBC cells

Concentration	%Viability
TritonX	100.02
PBS	1.92
25 µg/ml	46.18
50 µg/ml	34.05
100 µg/ml	30.65

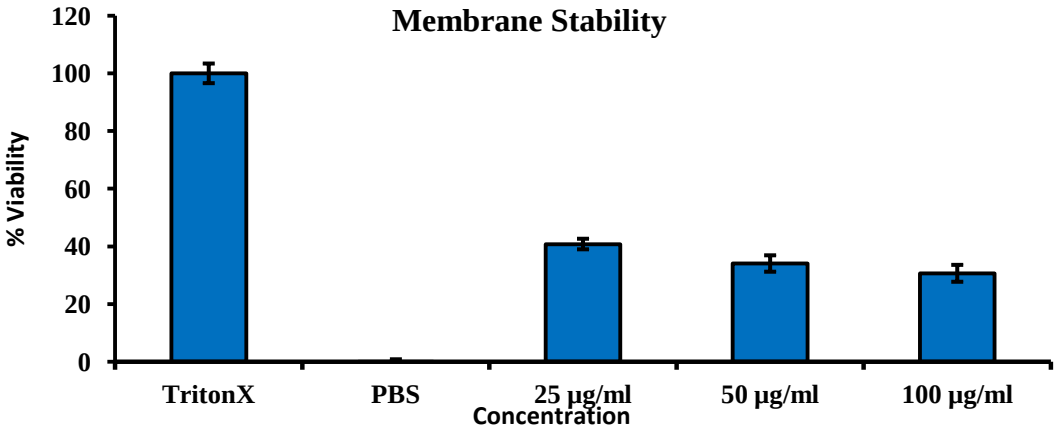


Fig.2.22 Graphical representation of membrane stability of dietary fibre on RBC cells

3.6. Haemolysis activity of dietary fibre on RBC cells

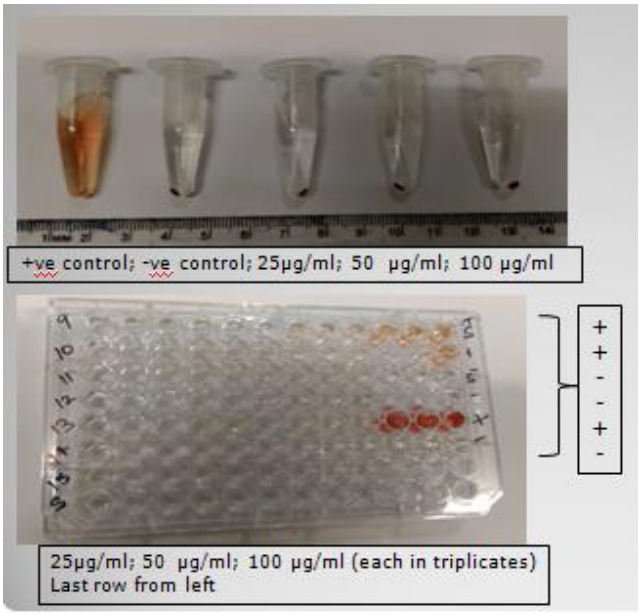


Fig.3.5 Haemolysis activity of dietary fibre on RBC cells

Table 3.2 Haemolysis activity of dietary fibre on RBC cells

Concentration	% Haemolysis
DW	100.00
PBS	17.58
25 µg/ml	15.77
50 µg/ml	15.21
100 µg/ml	17.82

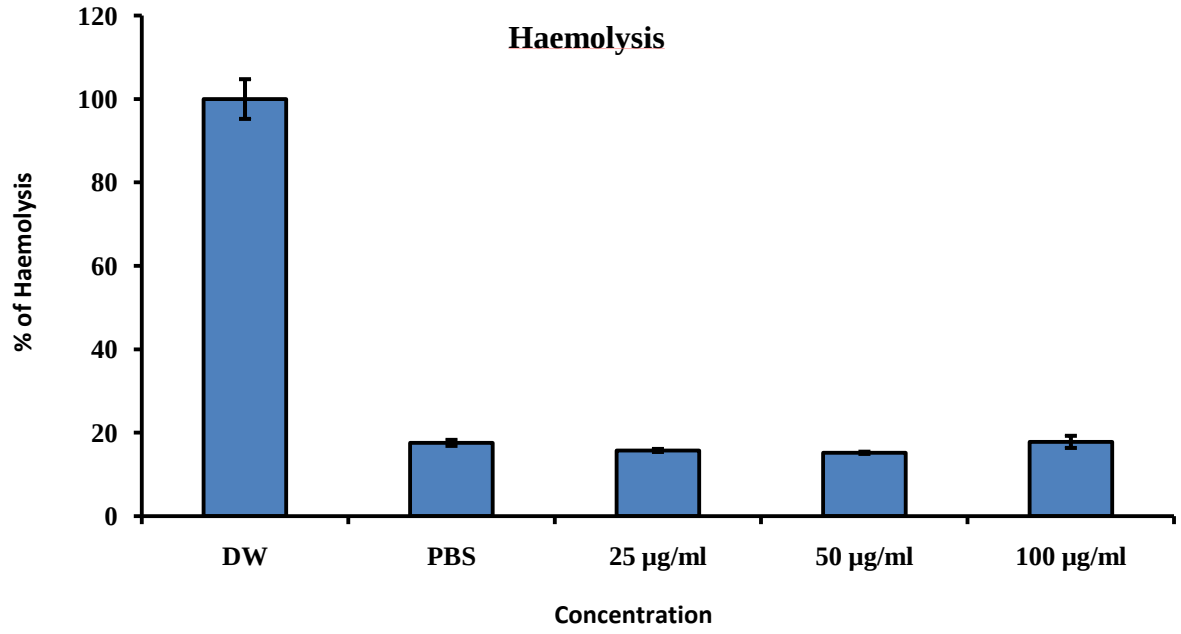


Fig.3.6 Graphical representation of haemolysis activity of dietary fibre on RBC cells

3.7. Cytotoxicity study on Human Peripheral Blood Mononuclear Cells (HPBMC)

Table 3.3 Viability percentage of Human Peripheral Blood Mononuclear Cells (HPBMC) on different concentrations of dietary fibre

Concentration (µg/ml)	Control	5	10	25	50	100
% Viability	100.00	91.18	94.66	97.80	93.55	91.52

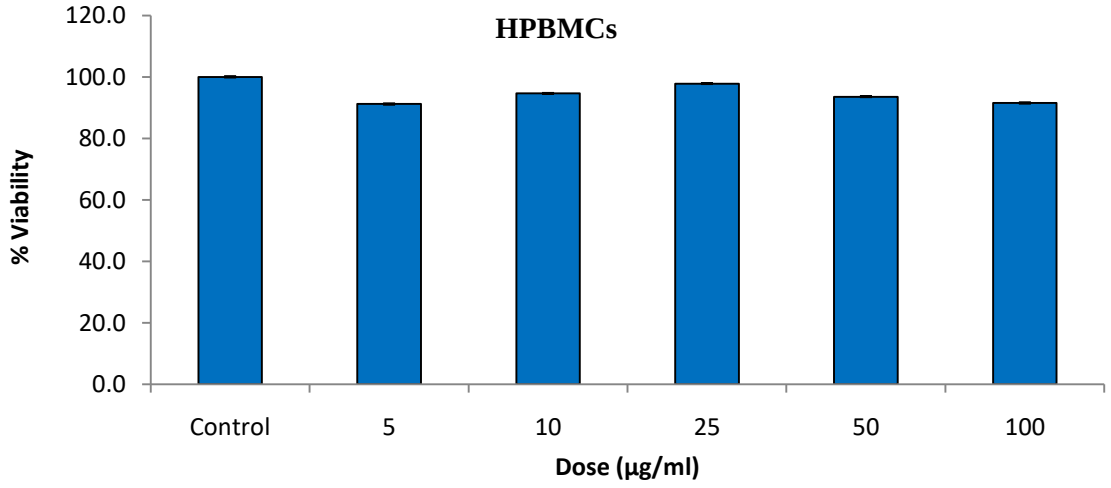


Fig.3.7 Viability percentage of Human Peripheral Blood Mononuclear Cells (HPBMC) on different concentrations of dietary fibre

3.8. MTT Assay of dietary fibre extract on HT-29 cells

Table 3.4 MTT Assay of dietary fibre extract on HT-29 cells

Concentration (µg/ml)	% Viability
Control	100.00
25	69.18
50	64.79
100	59.98
200	55.87

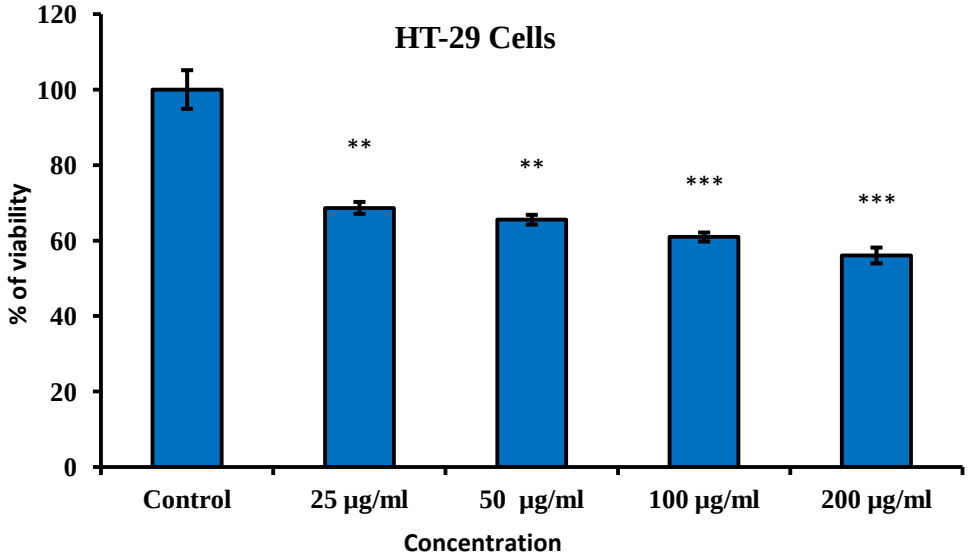


Fig.3.8 MTT Assay of dietary fibre extract on HT-29 cells

The extracted dietary fibre concentrate extracted from culinary banana flower (*Musa* ABB) showed overall nontoxic properties: negligible effect on cell membrane stability, non-haemolytic, HPBMs were viable at its different concentrations. The results indicated that the extracted dietary fibre concentrate were nontoxic towards peripheral blood mononuclear cell (PBMC). Also, with increase in the concentration of dietary fibre extract, %viability of the two human colon cell lines i.e. HT-29 cells were found to be decreased and possessed anticancer property as observed in HT-29 cancerous cell line.

Objective 4: Incorporation of dietary fibre concentrate extracted from culinary banana flower (*Musa* ABB) into food model and sensory evaluation

1. Cookies formulation and preparation:

Developed dietary fibre was incorporated in cookies as 1%, 3%, and 5% by substituting wheat flour. The formula used for making the cookies as follows - 100 g wheat flour , 50 g sugar , 25 g fat , 2 g baking soda and 25 ml water. Cookie dough was prepared according to the formula: 100g of flour (contain different proportion of dietary fibre and wheat flour), 50 g of sugar, 25 g of fat, 2 g of baking soda , 25 ml of water and various proportion of dietary fibre (other than control) to make required consistency of dough. The firm dough was rolled out to 3 mm thickness in a baking tray and cut into circles having 5 cm diameter with a cookie cutter. The cookies were placed on butter paper above aluminium tray and baked in a pre-heated oven at 175 ˚C for 15 min to produce cookies. Cookies sample were cooled and stored in air tight container (Jemziya et al., 2015).

Table 4.1 Cookies formulation and preparation

Ingredients (g)	Control	1% DF	3% DF	5% DF
Wheat flour	100	99	97	95
DF	0	1	3	5
Sugar	50	50	50	50
Fat/Butter	25	25	25	25
Baking soda	2	2	2	2
Water (ml)	25	25	25	25

DF = Dietary Fibre

Proximate analysis of developed cookies

The proximate composition of the cookies such as moisture, ash, fat, crude fibre, protein and total carbohydrate was measured by previously discussed methods (AOAC, 2010).

Physical analysis of cookies

The diameter of the cookies was measured by laying six cookies edge to edge and again the same set of cookies was rotated by 90º and the diameter was re-measured. Average values of the cookies were taken. The thickness of the cookies was measured by stacking six cookies one above the other and

dividing by the number of cookies gives the average thickness of each cookie. Spread ratio was calculated by dividing diameter by thickness (Ajila et al., 2008).

Total dietary fibre content of the cookies

The total dietary fibre was determined according to the AOAC enzymatic gravimetric method.

Antioxidant activity

Antioxidant activity was measured using the method described by Brand et al. (1995). Antioxidant activity was calculated as percent discoloration.

$$\text{Radical scavenging activity } (\%) = (\frac{1 - \text{As}}{\text{Ac}}) \times 100$$

Where, As= Absorbance of DPPH and extract

Ac = Absorbance of the control

Particle size distribution

The particle size distribution of cookies was done using particle size distribution analyzer equipped with dynamic light scattering (DLS) system (Microtrac Nanotrac Wave, MN401, USA). Deionized water was used for dispersion of the samples.

Color measurement

Hunter color measuring spectrophotometer was used for measurement of color. L*, a* and b* values of the cookies were measured for the determination of color, where L* indicates degree of lightness or darkness, if L* =0 then perfect black and if L*=100 then color is found to be perfect white; a* indicates degree of redness (+) and greenness (+) whereas b* indicates degree of yellowness (+) and blueness (-) (Pathare et al., 2013).

Physical and texture analysis of cookies

The physical characteristics of the cookies such as diameter, thickness, spread ratio varies with change in level of dietary fibre. Thickness slightly decrease and diameter slightly increase with increasing level of dietary fibre, which in turn increases the spread ratio of the cookies. This may be due to presence of high amount of insoluble fibre which forms network and having low water volume capacity.

Results and discussion

Physical and texture analysis of cookies

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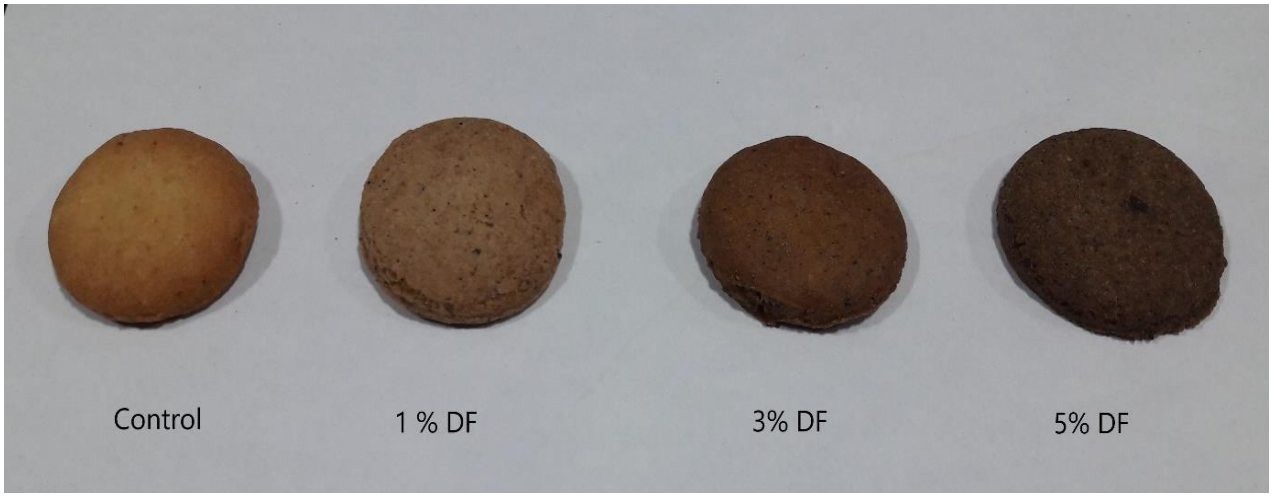


Fig.4.1 Cookies with different concentrations of dietary fibre

Table 4.2 Physical properties of cookies

Parameters	Control	1% DF	3% DF	5% DF
Diameter (mm)	26.10±0.20	26.20±0.17	26.43±0.06	26.46±0.11
Thickness (mm)	5.12±0.16	5.04±0.11	4.98±0.08	4.9±0.12
Spread ratio	5.09	5.18	5.30	5.40

Particle size distribution of cookies

The particle size distribution of 1% DF, 3% DF and 5%DF based on dynamic light scattering (DLS) are illustrated in the Figure.The mean particle size of 1% DF was less than 1µm and 5% DF was approximately 0.5 µm. More uniform and lesser particle size distribution with mean particle size less than 0.5 µm can be seen in the Figure compared to the 1% DF and 5% DF. Thus, the surface area increased as it depends on the size of particles. The increased surface area is responsible for improving functional properties.

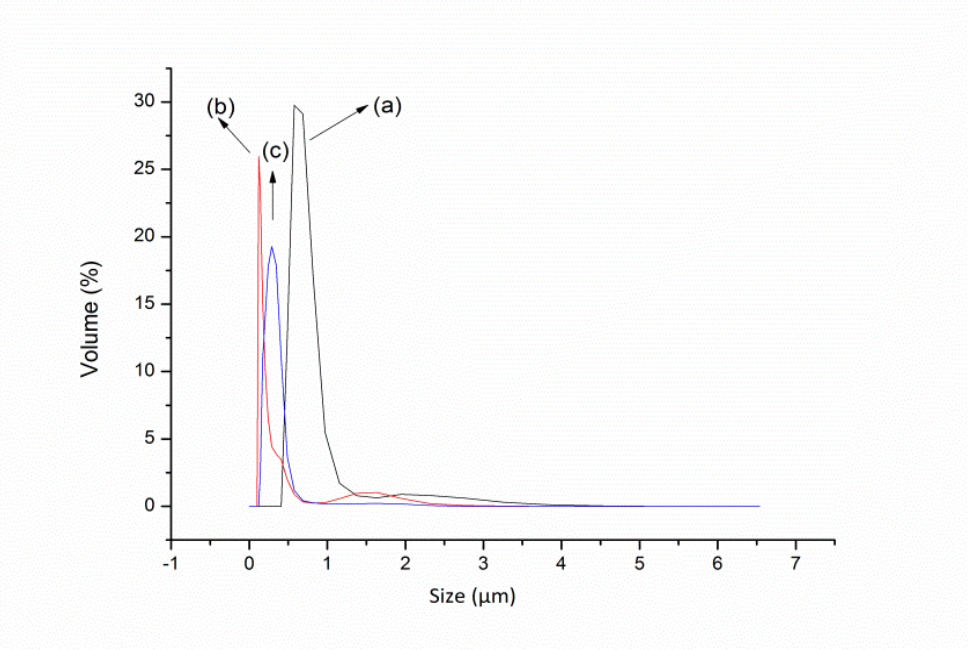


Fig. Particle size distribution of (a) 1% DF, (b) 3% DF and (c) 5% DF

Proximate composition of cookies

Table 4.3 shows the overall nutritional composition of cookies

Table 4.3 Proximate composition of cookies

Parameters (g/100g)	Control	1%	3%	5%
Moisture	5.08	5.12	5.21	5.23
Ash	1.08	1.21	1.25	1.27
Protein	6.82	6.39	5.78	5.15
Fat	18.36	18.09	17.72	17.7
Crude fibre	16.59	20.46	23.78	26.8
Carbohydrate	52.07	48.73	46.26	43.58

Antioxidant and radical scavenging potential of cookies

The incorporation of DF gradually increased the antioxidant properties in cookies with increasing concentration of DF (1% DF, 3% DF and 5%DF). Previous reports suggested that incorporation with dietary fibre improve the antioxidant properties of cookies. Radical scavenging potential of the cookies were measured in terms of DPPH radical scavenging activity. The incorporation of DF progressively increased the radical scavenging activity of the cookies. This may be correlated with the increase in DF content of the cookies. Thus, incorporation of DF improves the health benefits by increasing antioxidant activity and its dietary fibre content.

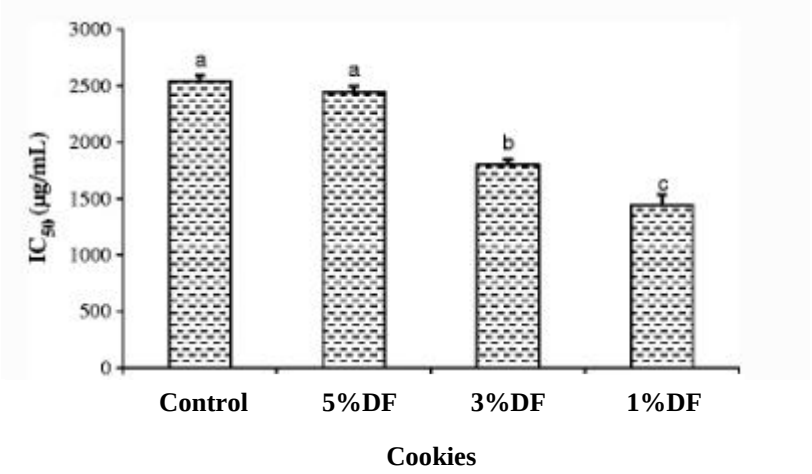


Fig. IC_{50} values of different Cookies for scavenging DPPH. Results are mean ± SD of triplicate measurements ($n = 3$) and the significance accepted at $P \leq 0.05$. Means with different letters are significantly different

Color measurement

Color of the cookies were measured by Hunter system using L*, a* and b* values. Table 23 showed that cookies became darker with increasing level of DF.

Table 4.4 L*, a*, b* values of cookies

Cookies	L*	a*	b*
Control	54.38±0.16	10.93±0.05	44.35±0.10
1 % DF	47.46±0.13	13.26±0.36	47.03±0.58
3% DF	34.11±0.81	21.87±0.62	57.2±0.85
5% DF	20.32±0.27	26.1±0.48	34.98±0.46

All data were mean ±SD of three replicates. DF= Dietary fibre.

2. Bread formulation and preparation:

The formulation used for control bread was: 100g refined flour, 4g sugar, 1.5g salt, 1.5g instant dry active yeast, 4g shortening, and 60g water. The selection of ingredients and quantity was determined after conducting preliminary experiments. Bread was developed by incorporating DF (2, 3 and 4%) with varying moisture content (64, 66 and 68%). Bread without incorporating DF and with 60% moisture content was prepared and coded as control. The dough formed after mixing all ingredients was proofed for 90 min at 30±2°C. Dough was moulded and placed in a lightly greased pan for final proofing for another 45 min. The bread dough was then baked at 220°C for 15 min. After baking was completed, bread loaves were immediately removed from pans and cooled to room temperature (30±2°C). The loaves were then sealed in polyethylene bags for further analysis. 9 batches of bread (Table 4.2) were prepared by incorporating varying DF (2-4% of flour mixture) and moisture (64-68%) using total mass and moisture balance (Himmelblau et al., 1967) as follows

$$X + Y = 170 \qquad (1)$$

$$X + a \times Y = b \times 170 \qquad (2)$$

Where, Y is flour mixture; X is the moisture in the dough; a is the moisture present in flour mixture and b is the moisture level (64-68%) added during dough preparation

Table 4.5 Batches (9) of bread with varying DF level and moisture

Sample code	Moisture (%)	DF (%)	DF (g)	Flour mix (g)	Water (g)
DF-A	64	2	1.37		
DF-B	64	3	2.06	68.76	101.24
DF-C	64	4	2.75		
DF-D	66	2	1.29		
DF-E	66	3	1.94	64.94	105.06
DF-F	66	4	2.59		

DF-G	68	2	1.22		
DF-H	68	3	1.83	61.12	108.88
DF-I	68	4	2.44		

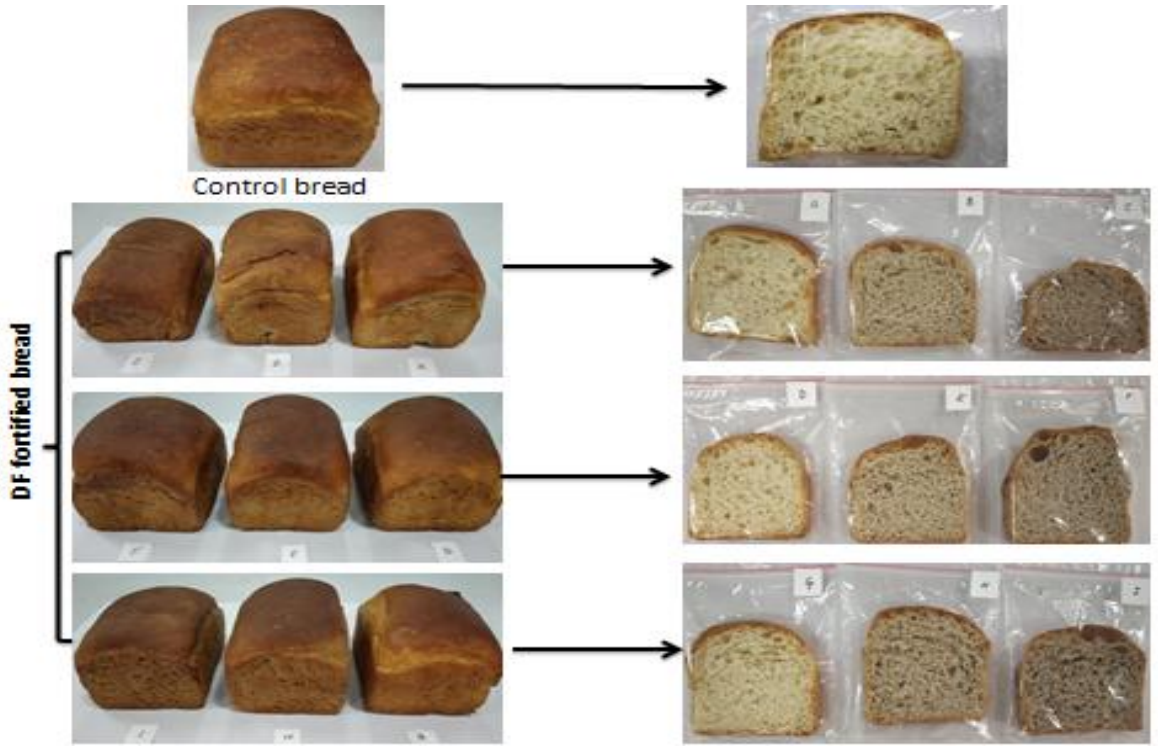


Fig.4.2 DF fortified breads along with the control bread

Dough rheology

Doughs rheological behavior was studied using rheometer (Anton Paar GmbH , MCR72, Austria) by geometry of parallel plate (25 mm diameter, 1.5 mm gap) and frequency sweep test was performed at 25° C. After setting the 1.5 mm gap, excess dough was removed and silicon oil was used to cover the exposed surface. Dough was rested for 10 min before measuring to reach thermal equilibrium. Then a frequency sweep test was done in the frequency ranged from 0.1 to 100 rad/sec at constant shear strain of 1%. Elastic modulus (G′), viscous modulus (G′′) and loss tangent ($tan\delta = \frac{G''}{G'}$) were attained for different frequency. Each sample was measured in triplicate using a fresh dough sample each time.

Chemical composition of bread

Bread chemical composition (moisture content, fat, protein, crude fibre, ash) were determined according to AOAC (2012). Carbohydrate content was calculated by differerence. Moreover, the soluble, insoluble and total DF content were determined following the enzymatic-gravimetric method (Prosky et al., 1988).

Physical properties of bread

i) Specific volume

Rapeseed displacement method [1] was used to determine specific volumes of bread samples using following equation

$$Specific\ volume\ (cm^3 g^{-1}) = \frac{loaf\ volume}{loaf\ weight}$$

ii) Water retention

Water retention of bread was calculated using the equation below

$$\%\ Water\ retention = \frac{w_a}{w_b} \times 100$$

Where, w_b is the weight of sample before baking; w_a is weight of sample after baking

iii) Texture profile analysis of bread crumb

The textural property of bread crumbs was determined according to the method explained by Alvarez et al. (2002). Texture analyzer (TA-HD plus, Stable Micro Systeme, UK) with 50 N load cell was used to determine the extensibility and firmness of bread samples. Bread sample (2 cm thickness) was taken from the centre of the slice. A cylindrical probe (25 mm diameter) was used to compress the bread samples 50% of their initial height at test speed of 1 mm/s. The peak force of compression was recorded as bread hardness or firmness. Springiness is the ratio of the height of sample springs back during the first compression compared to maximum deformation. Gumminess, springiness, chewiness and cohesiveness were determined as follows

Gumminess= $\left(\frac{A_2}{A_1}\right) \times Hardness,$

Springiness= $\frac{L_2}{L_1}$,

Chewiness= $Gumminess \times Springiness$

Cohesiveness= $\frac{A_2}{A_1}$,

Where, A_1 and A_2 are the area of first and second compression cycle, respectively; L_1 and L_2 are time difference of first and second compression cycle, respectively

Sensory evaluation

Sensory evaluation of bread samples were done immediately after bread loaves were cooled to room temperature (30±2°C). Bread loaves were sliced and sensory evaluation was performed using nine-point hedonic scale (Sudha et al., 2007). Bread was analysed on the basis of visual appearance, color, texture, taste, mouth feel and overall acceptability on 9 point hedonic scale (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much and 9-like extremely).

***In vitro* starch digestibility and Predicted glycemic index (pGI)**

In vitro starch digestion of bread was determined according to the procedure of AACC (2000) and Delgado-Andrade et al. (2010) with slight modifications. 1 g bread sample was mixed with 10 mL distilled water followed by addition of pancreatin-bile salt (2.5 ml) and 0.04 mL of amyloglucosidase and adjustment of pH 6.0 using $NaHCO_3$ (0.1 N). The mixture was then incubated at 37 °C for 3 h at 120 rpm. Aliquots were taken and mixed with ethanol at different times (0, 30, 90, 120, and 180 min) and the mixture was centrifuged at 1500g for 5 min. The hydrolyzed glucose content in the supernatants was calculated at 540 nm by GOPOD kit. The concentration of released glucose was plotted against each incubation time. Rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were determined from the above concentration-over-time curve at 30, 120 and 180 min, respectively.

The starch hydrolysis curve was obtained and the kinetics of starch hydrolysis follows first order kinetics as below

$$C = C_\infty(1 - kt)$$

Where, C and C∞ are concentration at time t and equilibrium concentration, k is kinetic constant and t is chosen time.

The area under curve (AUC) was determined from the concentration-over-time. Hydrolysis index (HI) was obtained from the ratio of AUC of each sample to the AUC of control bread. The HI was calculated for each sample using AUC as given in the equation below

$$AUC = C_\infty(t_\infty - t_0) - ({}^{C_\infty}/_{k})[1 - \exp[-k(t_\infty - t_0)]]$$

Where, C∞ is equilibrium percentage of hydrolyzed starch after 180 min, t_α is the final time (180 min), t_o is the initial time (0 min) and k is the kinetic constant.

The pGI was determined with the equation given below

$$pGI = 39.71 + 0.549HI$$

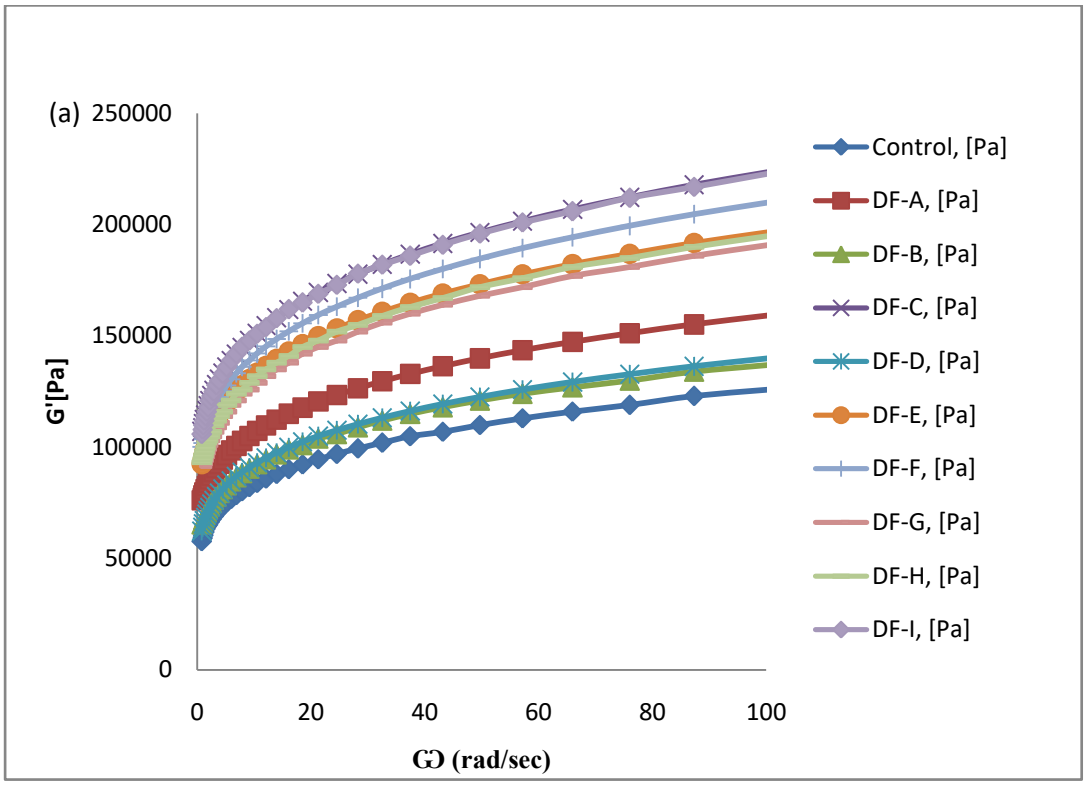
Statistical analysis

Experiments were carried out in triplicate. Means of data obtained were evaluated using Duncan's multiple range test to identify significant differences at the 0.05 probability ($p < 0.05$) using the SPSS 16.

Results and discussion

Dough rheology

The effect of DF addition on dough viscoelastic properties was analysed. The results revealed that elastic modulus (G′) and viscous modulus (G″) increased with angular frequency (ω) for all dough samples (Fig.4.3(a) and Fig.4.3(b)) and G′ is higher than G″, indicating solid behavior of the doughs. G′ and G″ were higher with increased incorporation of DF in dough, compared to control. The higher incorporation of DF enhanced the stiffness of dough as shown by higher G′ and G″ values compared to the control sample. The increases in modulus values were due to incomplete hydration of dough components (Navickis et al., 2010). The loss tangent (tan δ) (Fig.4.3(c)) was lesser with increased incorporation of DF compared to control dough, indicating a higher elasticity. Sample DF-D showed similar tan δ as control, depicting consistent contributions of the viscoelastic behavior of the dough. Dough consistency was increased by the addition of DF requiring increased water content in its formulation (Martinez et al., 2014). The effect of DF on the consistency and elasticity of dough could be due to DF remained unaltered in dough with a greater effect on dough structure which further affects the specific volume and hardness of bread. The increase of the dough consistency and elasticity was also observed after addition of potato, pea, bamboo fibres (Martinez et al., 2014).


(a)
250000
200000
150000
100000
50000
0
G'[Pa]
0
20
40
60
80
100
GO (rad/sec)
Control, [Pa]
DF-A, [Pa]
DF-B, [Pa]
DF-C, [Pa]
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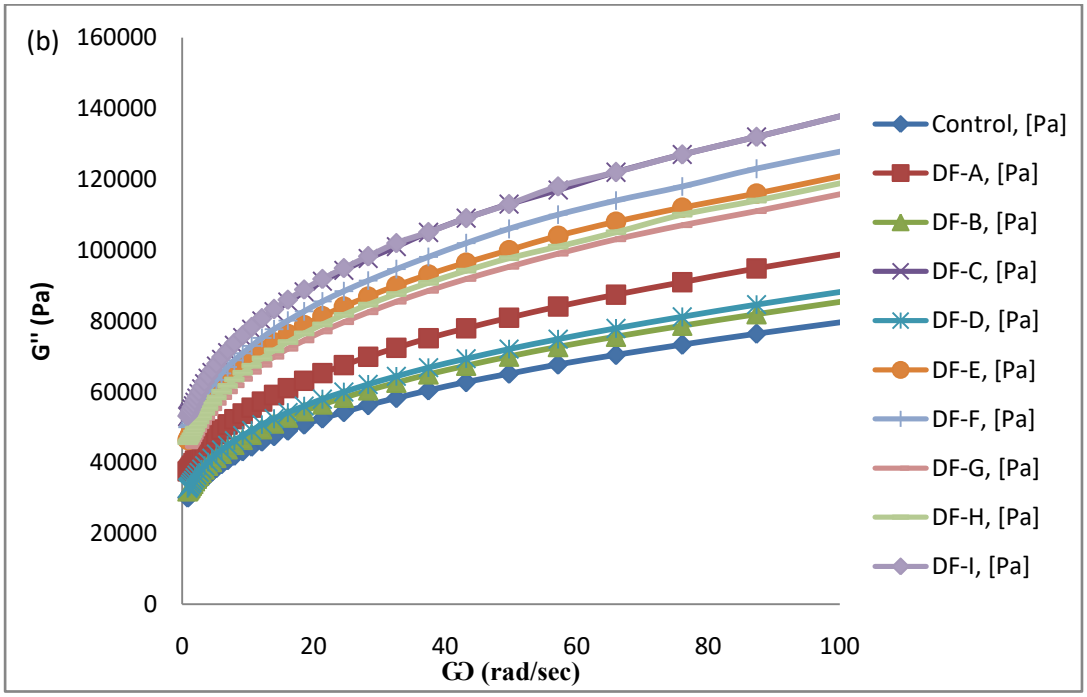
DF-E, [Pa]
DF-F, [Pa]
DF-G, [Pa]
DF-H, [Pa]
DF-I, [Pa]


(b)
160000
140000
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100000
80000
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40000
20000
0
G'' (Pa)
0
20
40
60
80
100
GO (rad/sec)
Control, [Pa]
DF-A, [Pa]
DF-B, [Pa]
DF-C, [Pa]
DF-D, [Pa]
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DF-H, [Pa]
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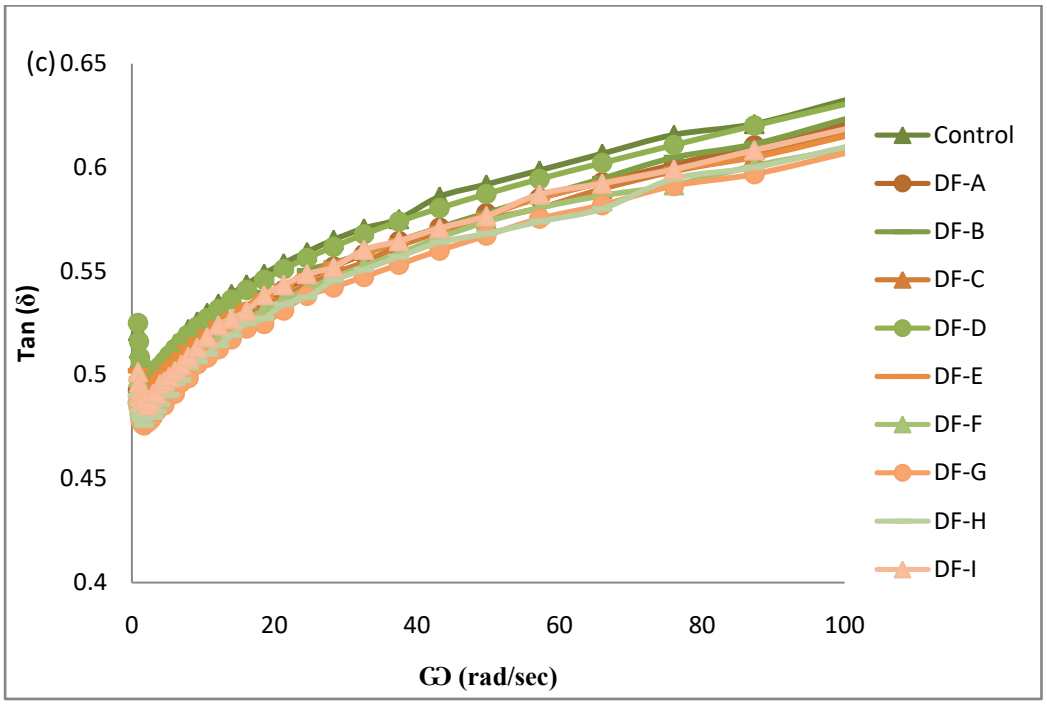


Fig.4.3 Changes in (a) storage modulus (G´), (b) viscous modulus (G´´) and loss tangent (tan δ) of control dough and DF-containing wheat dough

Chemical composition of DF fortified bread

The extracted DF from culinary banana bract using UAE (yield was 69.50%) contain moisture content (9.82 g/100g), fat (0.98 g/100g), protein (1.06 g/100g), ash (6.23 g/100g), starch (4.32 mg/100g), insoluble DF (78.73 g/100g), soluble DF (4.65 g/100g) and total DF (83.38 g/100g). Moisture content, crude fat, protein, crude fibre and DF content (soluble, insoluble and total DF) of bread samples (Fig.4.2) are illustrated in Table 4.6 (a). Results illustrated that the addition of DF in flour mixture of dough affects the chemical composition of bread. Moisture content varies from 30.28-35.27%, sample DF-C having lowest moisture content. The lipid content was in the range of 6.99-7.96 % and did not vary greatly among the bread samples. Protein content (8.46-10.08%) was the highest in control (10.08%) and the lowest in sample DF-C (8.46%) among other bread formulations. The addition of 4% DF affects the formation of gluten and might result in weak protein network in dough (Rossell et al., 2001). Ash content did not vary significantly among the bread samples and ranged from 1.85-2.03%. Carbohydrate content varied from 38.56-43.33 %. The variation in carbohydrate content was attributed to the difference in the contents of other constituents. Crude fibre and DF increased with the higher incorporation of DF (2-4%) with high total DF (19.65%) in sample DF-C (Table 4.6(b)).

Table 4.6(a) Chemical composition of bread samples

Sample code	Moisture (%)	Fat (%)	Protein (%)	Crude Fibre (%)	Ash (%)	Total Carbohydrate (%)
Control	35.27 ± 0.25^{i}	6.99 ± 0.02^{a}	10.08 ± 0.20^{f}	3.79 ± 0.18^{a}	1.85 ± 0.14^{a}	42.48 ± 1.03^{f}
DF-A	34.05 ± 0.05^{f}	7.89 ± 0.12^{c}	9.02 ± 0.10^{c}	7.46 ± 0.05^{b}	1.95 ± 0.32^{cd}	39.60 ± 0.16^{b}
DF-B	32.10 ± 0.10^{d}	7.83 ± 0.08^{c}	8.69 ± 0.16^{ab}	7.74 ± 0.17^{cd}	1.99 ± 0.12^{def}	40.83 ± 0.50^{d}
DF-C	30.28 ± 0.19^{a}	6.96 ± 0.07^{a}	8.46 ± 0.08^{a}	8.98 ± 0.08^{g}	1.99 ± 0.11^{def}	43.33 ± 0.13^{g}
DF-D	34.32 ± 0.20^{g}	7.56 ± 0.08^{b}	9.67 ± 0.15^{e}	7.57 ± 0.12^{bc}	1.92 ± 0.32^{bc}	38.56 ± 0.02^{a}
DF-E	32.89 ± 0.01^{e}	7.79 ± 0.11^{c}	8.56 ± 0.06^{ab}	7.85 ± 0.05^{d}	1.95 ± 0.01^{cde}	40.50 ± 0.11^{c}
DF-F	31.07 ± 0.11^{b}	6.91 ± 0.12^{a}	8.50 ± 0.05^{a}	8.58 ± 0.18^{f}	2.00 ± 0.90^{ef}	42.80 ± 0.14^{f}
DF-G	34.61 ± 0.12^{h}	7.78 ± 0.05^{c}	9.38 ± 0.15^{d}	7.72 ± 0.07^{cd}	1.88 ± 0.50^{ab}	38.83 ± 0.31^{a}
DF-H	32.94 ± 0.06^{e}	7.96 ± 0.11^{c}	8.75 ± 0.11^{b}	7.62 ± 0.10^{bc}	1.90 ± 0.36^{abc}	40.33 ± 1.00^{c}
DF-I	31.66 ± 0.10^{c}	7.55 ± 0.08^{b}	8.64 ± 0.07^{ab}	8.06 ± 0.06^{e}	2.03 ± 0.41^{f}	41.99 ± 0.67^{e}

*(Mean with different superscript letters in the same column represent a significant difference at $p<0.05$, values represent mean± SD; n=3)

Table 4.6(b) DF composition of bread samples

Sample Code	Insoluble DF (%)	Soluble DF (%)	Total DF (%)
Control	6.29 ± 0.08^{a}	0.23 ± 0.04^{a}	6.53 ± 0.03^{a}
DF-A	15.05 ± 0.47^{b}	0.73 ± 0.03^{b}	15.92 ± 0.03^{b}
DF-B	16.83 ± 0.74^{e}	0.87 ± 0.02^{d}	17.92 ± 0.08^{g}
DF-C	18.42 ± 0.52^{g}	1.00 ± 0.11^{f}	19.65 ± 0.41^{i}
DF-D	15.48 ± 0.08^{c}	0.72 ± 0.42^{b}	16.57 ± 0.09^{d}

DF-E	16.53±0.06^{d}	0.79±0.01^{c}	17.27±0.03^{e}
DF-F	18.07±1.13^{f}	0.98±0.01^{f}	19.24±0.10^{h}
DF-G	15.00±0.94^{b}	0.69±0.02^{c}	16.12±0.07^{c}
DF-H	16.62±0.10^{d}	0.86±0.31^{d}	17.57±0.04^{f}
DF-I	18.04±077^{g}	0.93±0.02^{e}	19.20±0.07^{h}

*(Mean with different superscript letters in the same column represent a significant difference at $p<0.05$, values represent mean± SD; n=3)

Physical properties of DF fortified bread

1. Water retention and specific volume

The water retention ranged from 83.22-87.01% (Table 4.6 (c)), indicated a non-uniform absorption of water among the dough samples. Water retention decreased with the increased incorporation of DF (2-4%). High DF addition competes with gluten for water molecules, resulting non-sufficient water for stretching of gluten present in dough (Navickis et al., 2010). Specific volume is an important quality characteristics of bread as it refers to inflating ability of dough (Giannou et al., 2007). Specific volume varied from 5.46-5.83 cm^3/g (Table 4.6 (c)) with control having the highest specific volume (5.83 cm^3/g) which did not differ significantly from sample DF-D and sample DF-G. As the DF level in bread flour increased to 4%, the specific volume was successively decreased to 5.46 cm^3/g. Incorporation of DF might result in gluten dilution effect, leading to lower bread loaf volume. Reduction in the specific volume of bread was reported for bread fortified with wheat fibre, psyllium husk fibre and partially hydrolyzed guar gum (Park et al., 1997; Mudgil et al., 2016).

Table 4.6(c) Water retention (%) and specific volume (cm^3g^{-1}) of bread samples

Sample code	Water retention (%)	Specific volume (cm^3g^{-1})
Control	87.01±0.10^{f}	5.83±0.02^{e}
DF-A	86.49±0.02^{e}	5.71±0.01^{d}
DF-B	85.19±0.12^{c}	5.52±0.02^{b}

DF-C	83.22±0.24[a]	5.46±0.01[a]
DF-D	86.76±0.01[ef]	5.83±0.00[e]
DF-E	84.91±0.03[c]	5.62±0.01[c]
DF-F	84.06±0.05[b]	5.49±0.01[b]
DF-G	86.4±0.25[e]	5.83±0.02[e]
DF-H	85.68±0.49[d]	5.46±0.05[a]
DF-I	84.44±0.48[b]	5.51±0.01[b]

*(Mean with different superscript letters in the same column represent a significant difference at $p<0.05$, values represent mean± SD; n=3)

2. Texture profile analysis of bread crumb

The incorporation of different level of DF on bread flour impacts the textural properties of bread crumb (Table 4.6 (d)). The hardness varied significantly (1.32-5.24 N) among samples and control having the lowest hardness (1.32 N) followed by sample DF-D. The significant increase in hardness value for bread fortified with 4% DF was due to gluten dilution effect which interferes with gas retention ability in gluten network. Thereby, leading to tight structure of bread crumb (Sui et al., 2016). Springiness varied 0.66-0.91, control having the highest springiness which is significantly similar to sample DF-D and DF-G. The lower springiness with 4% DF is due to weak gluten structure with lower expansion of dough. Chewiness ranged from 0.64-1.95 N and it is directly proportional to hardness. Low cohesiveness was found for sample DF-C (0.36), indicating the reduction of strength of internal bond between bread ingredients (Valcarcel-Yamani et al., 2013). Sample DF-D showed improved textural properties in terms of lower hardness, high springiness next to control. Several studies stated that the addition of inulin increased breadcrumb hardness and chewiness which is in agreement with our results (Poinot et al., 2010; O'Brien et al., 2003; Wang et al., 2002).

Table 4.6(d) Texture profile analysis of bread samples

Sample code	Hardness (N)	Gumminess (N)	Springiness	Chewiness (N)	Cohesiveness
Control	1.32±0.02^{a}	0.70±0.01^{a}	0.91±0.01^{g}	0.64±0.02^{a}	0.55±0.03^{f}
DF-A	3.83±0.01^{g}	1.79±0.06^{f}	0.88±0.02^{f}	1.61±0.05^{g}	0.46±0.02^{c}
DF-B	2.41±0.36^{d}	1.31±0.10b^{d}	0.84±0.01^{de}	0.98±0.03^{d}	0.47±0.02^{cd}
DF-C	3.20±0.15^{f}	1.20±0.02^{cd}	0.66±0.01^{a}	0.82±0.02^{c}	0.36±0.01^{a}
DF-D	1.7±0.12^{b}	0.78±0.01^{a}	0.91±0.01^{g}	0.72±0.01^{b}	0.49±0.02^{d}
DF-E	3.17±0.20^{f}	1.55±0.02^{e}	0.85±0.01^{e}	1.32±0.10^{f}	0.47±0.02^{cd}
DF-F	2.7±0.08^{e}	1.22±0.02^{cd}	0.82±0.01^{c}	1.04±0.10^{e}	0.46±0.02^{c}
DF-G	2.18±0.06^{c}	1.14±0.16^{c}	0.89±0.01^{fg}	1.05±0.02^{e}	0.49±0.02^{de}
DF-H	2.41±0.15^{d}	0.94±0.02^{b}	0.72±0.02^{b}	0.68±0.01^{ab}	0.39±0.02^{b}
DF-I	5.24±0.10^{h}	2.30±0.49^{g}	0.83±0.10^{cd}	1.95±0.01^{h}	0.41±0.01^{b}

*(Mean with different superscript letters in the same column represent a significant difference at $p<0.05$, values represent mean± SD; n=3)

Sensory evaluation

Sensory analysis for three different cookies with 1%, 3%, and 5% of dietary fibre concentrate extracted from culinary banana flower (*Musa* ABB) and the control were done. Sensory scores with five different attributes viz., color, texture, taste, flavor and overall acceptability are shown in Table 4.7.

Table 4.7 Sensory score of cookies

Parameters	Control	1% DF	3% DF	5% DF
Color	7.12±0.24	7.5±0.21	6.07±0.16	6.30±0.07
Texture	6.84±0.59	6.07±0.67	6.58±0.94	6.42±0.13
Taste	7.51±0.17	6.87±0.16	6.46±0.04	6.97±0.36
Flavor	6.92±0.33	6.79±0.48	7.82±0.52	7.06±0.41
Overall acceptibility	7.04±0.46	6.72±0.27	6.95±0.71	6.74±0.65

From the above Table 4.7, we can observe that the different parameters values of the developed products with different percentage of dietary fibre concentrate. The overall acceptability of the developed products was found not to be highly different from each other. The product with 3% dietary fibre concentrate with 6.95±0.71 points was reported to have the highest overall acceptability, followed by 5% dietary fibre concentrate (6.74±0.65 points) and 1% dietary fibre concentrate (6.72±0.27 points).

Furthermore, sensory analysis as shown by radar chart (Fig. 4.4) indicates that sample DF-D (8.16) with high overall acceptability next to control bread (8.66). The increased level of DF addition (4%) in the flour mixture influences the sensory attributes negatively with poor overall acceptability due to the hard texture and specific taste of DF. Hence, addition of 2% DF with 66% moisture can be successfully used for making DF fortified bread with high consumer acceptability. Mudgil et al. (2016) reported that PHGG (partially hydrolysed guar gum) supplementation at 1.59% concentration with 63.54 % moisture resulted bread with higher quality and overall acceptability, not significantly different from control. As reported by Rubel et al. (2015) DF (inulin-rich carbohydrate) enrichment at the level of 2.5% produced bread with acceptable sensory attributes in terms of taste, color, sponginess and texture. A further increase in the level of DF (upto 5%) results in unacceptable quality bread.

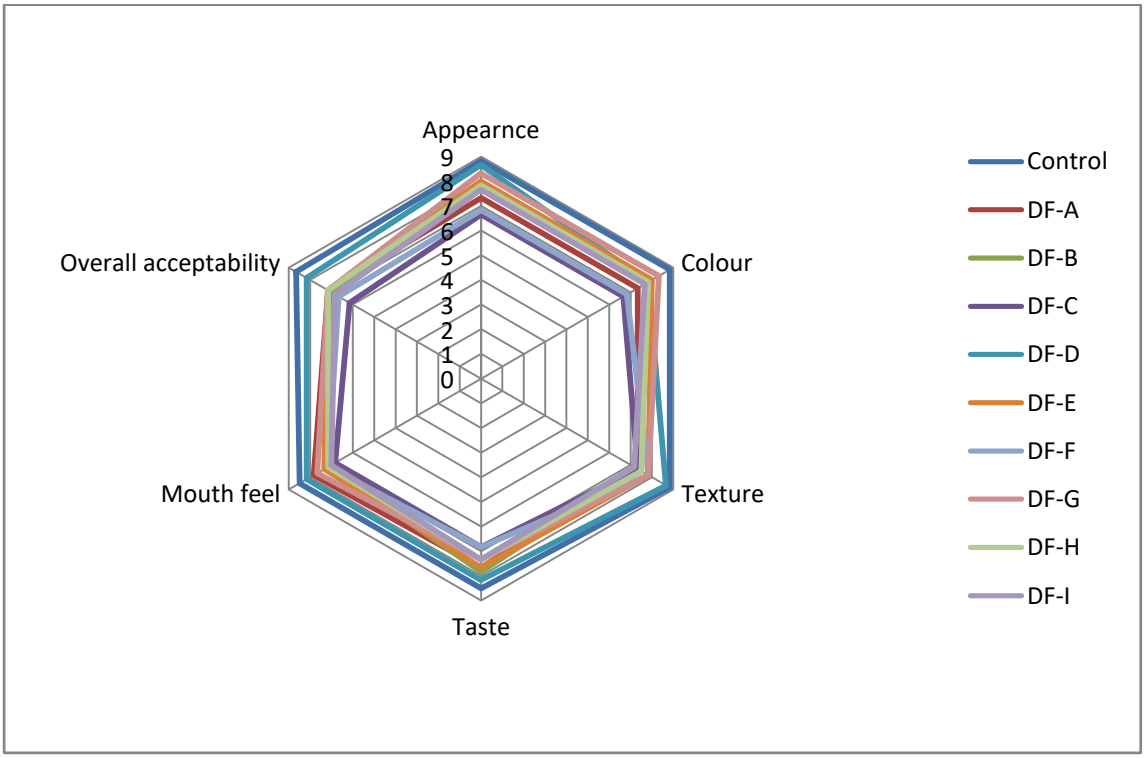


Fig.4.4 Radar chart of DF fortified bread

In vitro starch digestibility

The glucose release curve of bread samples are displayed in Fig.4.5. The kinetics of starch hydrolysis follows first order kinetics. The starch digestible fractions were determined and are shown in Table 4.8. The rate of digestion in first 30 min and from 30 to 120 min was considered as RDS and SDS, respectively and it indicates the rate of absorption in small intestine. The RDS content decreased with the increased level of incorporation of DF, sample DF-C showing the lowest RDS (33.72 mg/g sample). However, increasing the level of DF in bread flour mixture, the SDS content was successively increased 41.76 mg/g sample for 4% DF which was significantly (P<0.05) higher than control bread. SDS is more favourable over RDS as it reflects a gradual increase in postprandial glucose and controlling the blood glucose level (Jenkins et al., 1978). Resistant starch content varied with a range of 4.29-12.44 mg/g sample. Addition of insoluble DF tended to decrease RDS and increase SDS in fibre-enriched cakes compared to control (Oh et al., 2014).

pGI values were calculated for each of the bread sample and are presented in Table 4.8. The pGI values of DF fortified breads decreased significantly (p<0.05) from control. The lowest pGI value was obtained for sample DF-C (78.36). The increased level of DF inhibit starch digestion significantly which is related to its high water holding capacity, resulting lower pGI value (Huang et al., 2008). Lower carbohydrate digestibility with lower GI value obtained for pasta and extruded cereal foods with added inulin as a source of soluble DF (Brennan et al., 2004; Brennan et al., 2008). Moreover, insoluble DF is more effective than soluble DF in lowering the pGI value as reported by Oh et al. (2014).

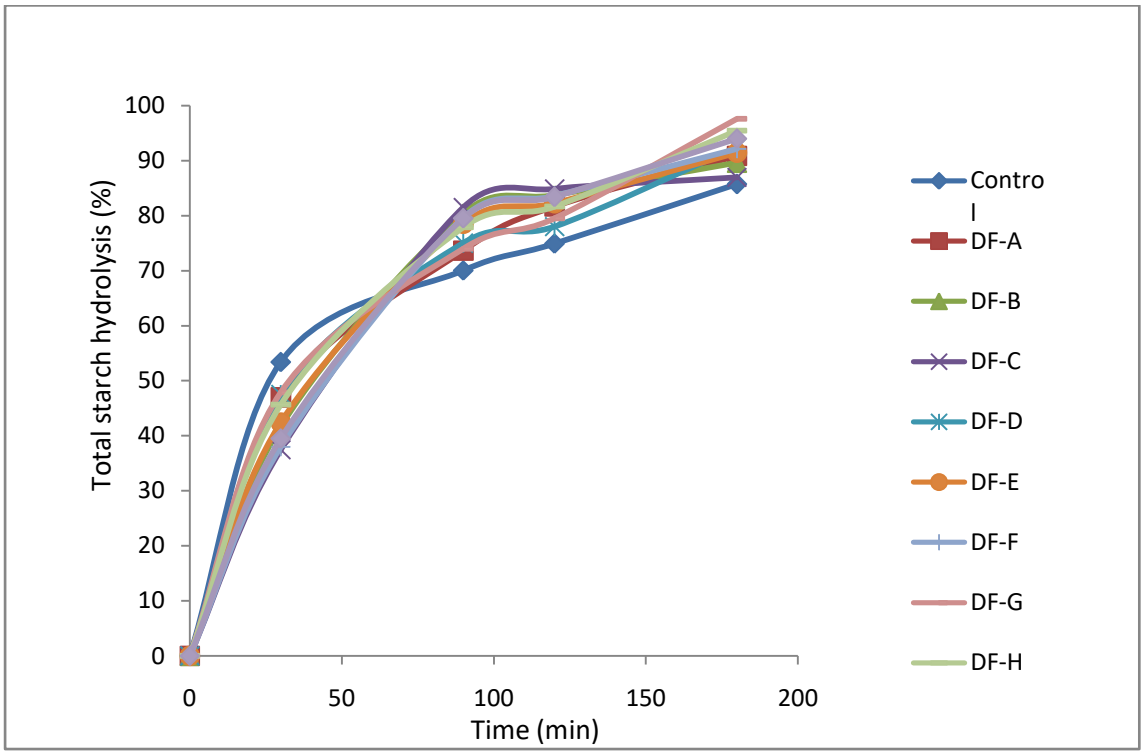


Fig.4.5 Rate of total starch hydrolysis of bread samples

Table 4.8 Starch digestible fraction and pGI of bread samples

Sample code	RDS (mg/g sample)	SDS (mg/g sample)	RS (mg/g sample)	pGI
Control	48.09±0.83^{f}	24.77±0.64^{a}	4.29±0.87^{a}	94.62±0.05^{h}
DF-A	42.30±1.00^{de}	33.03±1.04^{d}	6.48±0.61^{c}	88.43±0.14^{e}
DF-B	37.53±1.52^{c}	37.21±0.73^{f}	6.24±0.61^{c}	81.46±0.24^{b}
DF-C	33.72±0.28^{a}	41.76±0.55^{i}	9.37±0.89^{g}	78.36±0.10^{a}
DF-D	42.79±0.50^{e}	31.05±0.80^{c}	5.34±0.76^{b}	88.99±0.05^{f}
DF-E	38.16±0.76^{c}	35.91±0.74^{g}	8.27±1.26^{e}	81.33±0.33^{b}
DF-F	34.20±1.25^{ab}	41.02±0.79^{g}	7.77±0.50^{d}	82.96±0.23^{c}

DF-G	43.11±0.89^{e}	28.49±0.63^{b}	6.29±0.64^{c}	93.31±1.3^{g}
DF-H	41.09±0.61^{d}	32.40±1.15^{e}	12.44±0.92^{g}	86.02±.41^{d}
DF-I	35.55±1.35^{b}	39.51±0.56^{h}	9.55±0.82^{f}	82.71±0.17^{c}

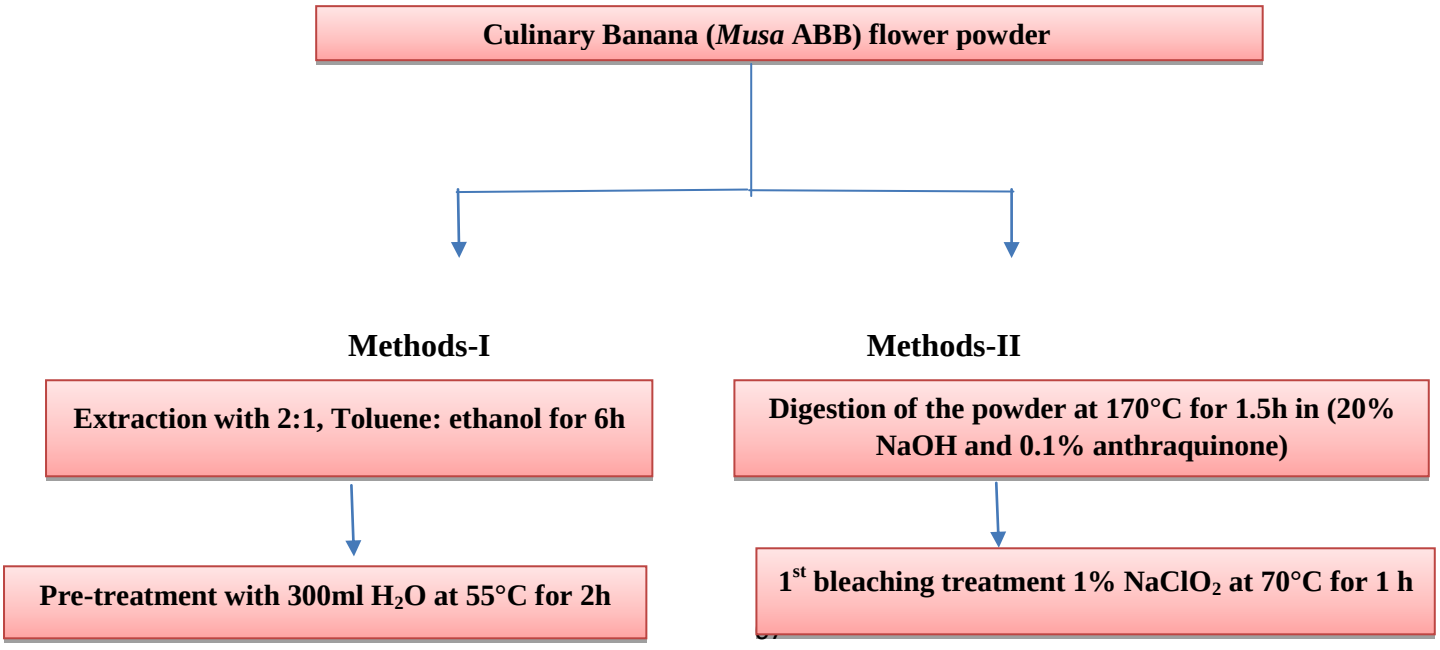
*(Mean with different superscript letters in the same column represent a significant difference at $p<0.05$, values represent mean± SD; n=3)

The incorporation of different level of DF (2-4% of flour mixture) with varying moisture (64-68%) affects the dough rheological behavior. It also has significant impact on the attributes of bread, its starch digestibility and pGI. The increased incorporation of DF (2-4%) resulted in enhancement of stiffness, consistency and elasticity of dough as indicated by higher G´. The highest total DF was obtained with higher incorporation of DF (4% of flour mixture). Increasing DF level (2-4%) significantly decreased specific volume and produced firmer bread with high chewiness and less springiness as evident from texture analysis data. Therefore, incorporation of DF (2% of flour mixture) with 66% moisture level was chosen as it produced nutritionally balanced bread and not significantly different from control (higher specific volume, lower hardness, high springiness). Besides, it had acceptable sensory attributes in terms of color, taste, texture and appearance.

Objective 5: Preparation of biopolymer based nanocellulose fiber and its application in film

Materials and methods

5.1 Flow chart for the two different steps involved in cellulose extraction



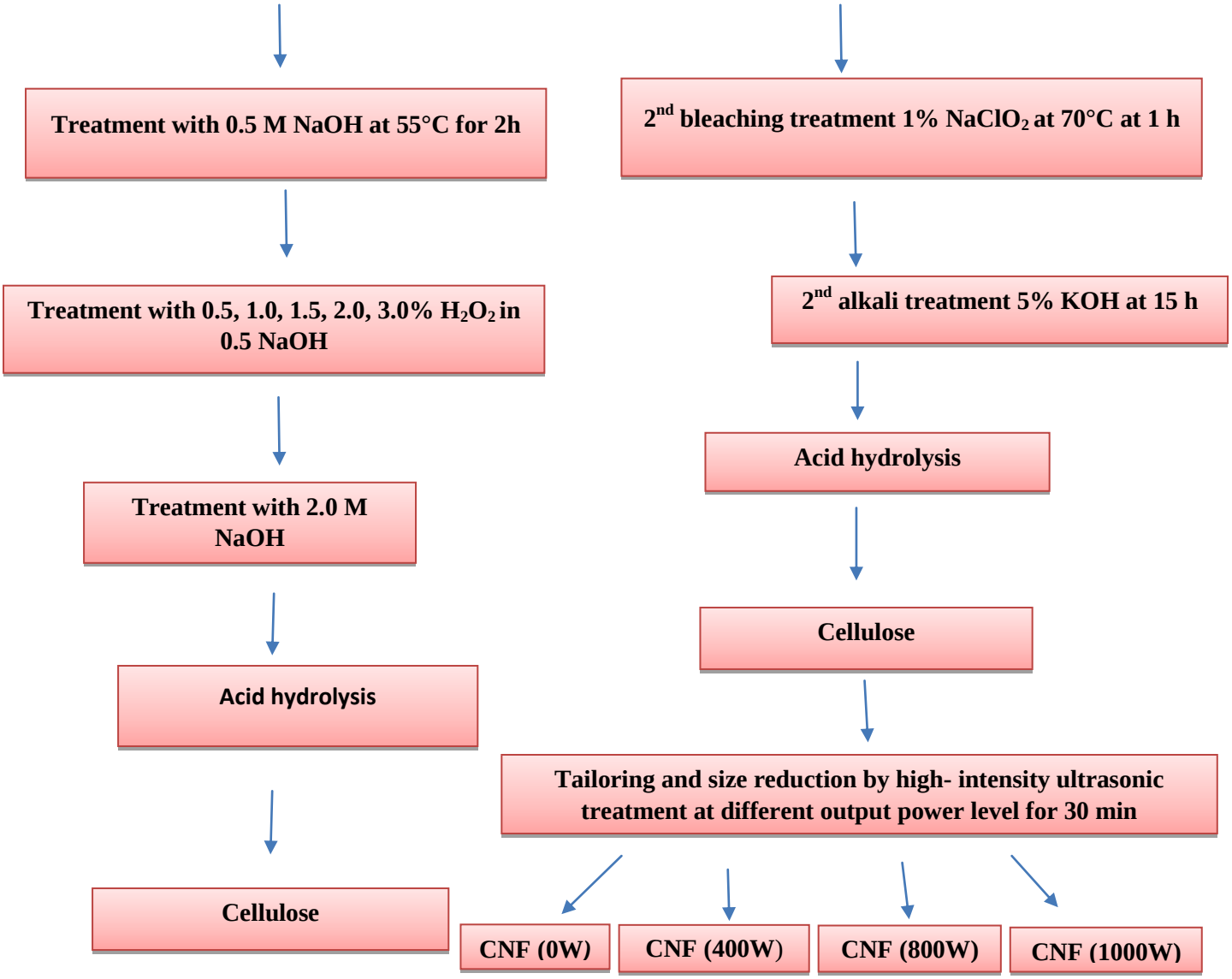


Fig. 5.1 Detailed flow chart for the two different steps involved in cellulose extraction

5.2. Preparation of banana flower powder

Fresh banana flower after harvest was taken and immediately cut pieces 0.3 × 2.5 cm in size and dried at 55ºC for 10 h until reaching a moisture content of 7.5 % (dry basis) using a tray dryer. After cooling to room temperature, the banana flower was weighed, ground, and passed through a 35 mesh sieve before sampling for its chemical composition analysis (AOAC, 2010) and it was then kept in a polyethylene plastic bag and placed in a refrigerator at 4ºC for analysis.

5.3. Extraction (Method–I) (Elissari et al., 2014)

5.3.1. Dewaxing of the powder

The cut sample was ground to pass a 1.0 mm size screen. The dried powder was first extracted with toluene: ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h and the dewaxed meal was allowed to dry in an oven 60ºC for 16 h.

5.3.2. Isolation of cellulose

The culinary banana flower was first given alkali treatment by cooking in a digester (Kelplus, Pelican Equipment, India) with a solution of 20% (w/v) sodium hydroxide and 0.1% anthraquinone with bran to solution ratio of 1:20 at 170ºC for 1h 30 min which partially solubilize the pectin lignin and hemicellulose. The digested bran was washed in distilled water to remove pectin, lignin and hemicellulose. The soluble pellets remained after first alkali treatment was further dignified with 1% sodium chlorite at pH 5 adjusted with 10% V/V acetic acid at 70ºC for 1 h. This was the first bleaching process which broke down the phenolic compounds. The bleaching treatment also leached out byproduct of such breakdown therefore whitening the pulp after each treatment. A second bleaching treatment was given to maintain the same condition to the insoluble matter after first bleaching treatment. A second alkali treatment was given using 5% KOH at room temperature for 15h to neutralize the pellets after second bleaching treatment. The residual hemicellulose leached out after second alkali treatment, which is then washed from the pellets. Finally the insoluble pellets were given acid hydrolysis which solubilize the minerals and the amorphous cellulose. After each treatment, insoluble minerals, amorphous cellulose and insoluble pellets were washed with double distilled water until it was neutralized using centrifuge (6000 rpm for 30 min).

5.4. Extraction (Method –II)

Isolation of cellulose was done using alkaline with ultrasonic treatment. The de waxed SCB (40g) will be sequentially treated with 300ml H_2O at 55ºC for 2h with or without first ultrasonic irradiation for 40 min, 0.5 M NaOH, 0.5%,1.0%,1.5%, 2.0% and 3.0% H_2O_2 in 200 ml 0.5 M NaOH, and 200ml 2M NaOH at 55ºC for 2h. The insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrated was neutral, then dried at 60ºC.

During the alkaline treatment, the alkaline solution hydrolyzed constituents like starch pectin and hemicelluloses. As for the bleaching treatment, hydrogen peroxide were remove lignin and tannins, which are responsible for the brown color of the banana flower. This is the most widespread technique to

remove lignin from plants at the laboratory scale. During the bleaching treatment, chlorine, peroxide and chlorites will rapidly oxidize lignin, generating hydroxyl, carbonyl group; these groups will facilitate lignin solubilization in alkaline medium and thus cellulose purification (Dufrene et al., 1997).

During the alkaline treatment, the alkaline solution was hydrolyzer constituents like starch, pectin, and hemicelluloses. The digested peel was washed with distilled water to remove those pectin, lignin and hemicelluloses. The insoluble pellets remained after the first alkali treatment was further dignified with 1% (w/v) sodium chlorite (at pH 5 adjusted with 10% v/v acetic acid) at 70º C for 1 h. The insoluble pellets remained after first bleaching treatment was washed and second bleaching treatment was given again maintain the same condition as in this first bleaching process which resulted in further effective discoloration and confirming the leading out of phenolic compounds and lignin. The neutralized insoluble pellets remained after second bleaching process were subjected to second alkali treatment with 5% (w/w) KOH solution at ambient temperature (25±2ºC) for 15 h followed by washing which helped in elimination of residual hemicellulose. Finally, the insoluble pellets were given to acid hydrolysis treatment with solution of 1% (v/v) sulfuric acid at 80ºC for 1 h. The acid hydrolysis treatment the insoluble pellets were washed with double distilled water and centrifuged at 10,000 rpm at 4ºC for 20 min until the pellets were neutralized. The pellets were kept in water-swollen state during whole chemical process in order to avoid generating strong hydrogen bonding among nanofibers.

The chemically purified cellulose fibers were soaked in deionized water (concentration~0.5 % mass) and subjected to tailoring and size reduction by giving high-intensity ultrasonic treatment. The solution of chemically purified cellulose fiber (120 ml) was placed in ultrasonic generator (UW 2070, Bandelin sonoplus) having 1.5 cm (diameter) cylindrical titanium alloy probe tip at frequency of 25 kHz. Ultrasonication was conducted at 400,800 and 1000W individually to characterize the effect of ultrasonic intensity on nano fibrillation of chemically purified cellulose. The ultrasonic treatment was carried out in ice water bath and ice was maintained throughout the sonication time. The detailed steps followed to obtain cellulose nanofibers are illustrated. The obtained nanofiber were freeze dried (LDF-5512, Daihan labtech Co.,south korea) and stored at 4ºC in sealed containers.

Fig. 5.2 High intensity ultrasonication (HIUS)

5.5. Purity of cellulose

Cellulose is the most abundant organic compound in nature. It is the major structural polysaacharide in plant cell wall, made up of D-glucose units which are linked to each other by ß-1-4- glycosidic bond. It is the major content of the farm waste. It was done standard method of cellulose estimation given in Thimmaiah et al. (2013) by dissolving in acid and then measuring in spectrophotometer with anthrone as coloring agent.

5.6. Synthesis of nanocellulose

High intensity ultrasonication can be considered as a mechanical method for producing cellulose nanofiber with hydrodynamic forces (Cheng et al., 2009). In the process, ultrasonic waves create strong mechanical stress because of cavitation and therefore cause to disaggregation of cellulose fiber to nanofiber oxidized fiber treatment with ultrasonic probe is more efficient for nanocellulose production than other methods.

5.7. Characterization of cellulose and nanocellulose

5.7.1. TEM

The sample was analyzed using TEM to have the length and diameter of the nano cellulose formed after chemical and ultrasonic treatment of the cellulose. The aspect ratio, length and breadth of the nano fiber

were calculated using this technique. The sample was dispersed in water and reading was taken at 2000nm scale and 14000X (scale bar 2,000 nm) using 12000V.

5.7.2. Preparation of the soluble dietary fiber and nano fibrillated cellulose film

Aqueous solution of nanocellulose (5ml) was filtered in vacuum filter and then it was pressed in a hot press heating up to temperature 2,400°C (4.350°F) , pressure of up to 50 MPa (7300 psi) and then kept 5 min for oven drying at 93ºC in a vacuum oven.

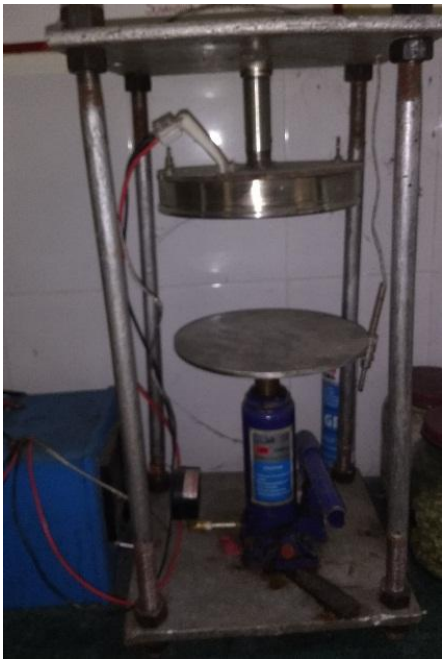


Fig. 5.3 Hot press

5.8. Characterization of soluble dietary fiber and nano fibrillated cellulose composite film

5.8.1. SEM (Scanning Electron Micrograph)

Scanning electron microscope was used to investigate the microstructure of film obtained after undergoing different chemical and ultrasonic treatments. Sample were placed in metal stud using double sided tape and coated with a fine layer of gold using a sputter gold coater. Sample micrographs were observed at a magnification of 1000x (scale bar 10µm) at an acceleration.

5.8.2. Film Thickness

The average thickness of 10 pieces of the film stacked one above another was measured by using a micrometer (Alton M820-25, China) with a sensitivity of 0.01 mm.

5.8.3. Swelling

Films were cut into 1×2 cm in size and measured the weight (W1). Films were immersed in deionized water (23 °C) for 2 min. Wet samples were wiped with filter paper to remove excess liquid and weight of the sample was noted (W2). The swelling occurs due to water absorption which was calculated as

$$\text{Swelling\%} = \frac{W1-W2}{W1} \text{ x } 1000$$

Where,

W_1 - Initial weight

W_2 - Weight of wet sample after immersing in deionized water

5.8.4. Solubility in water

Solubility in water was defined as the percentage of the dry matter of film which is solubilized after 24 h immersion in water. Film specimens were kept in a desiccator containing dry calcium sulphate ($CaSO_4$) till they reached constant weight. Afterwards, about 500 mg of each film were immersed in beakers containing 50 ml of distilled water at 23 °C for 24 h with periodical gentle manual agitation. The films were removed from the water and were placed back in the desiccator until they reached a constant weight to obtain the final dry weight of the film. The percentage of the total soluble matter (%TSM) of the films was calculated using:

$$\text{TSM} = \frac{Initial\ \ dry\ \ weig\,ht - Final\ \ dry\ \ weig\,ht}{Initial\ \ dry\ \ weig\,ht} \times 100$$

5.8.5. Tensile Properties

Tensile strength (TS) and elongation at break (EB) of films were measured using Texture Analyzer (TA-HD Plus, Stable Microsystems, UK). Kieffer Dough and Gluten Extensibility Rig (A/KIE) was used for this test. The thin strips (5×1 cm) were cut from each film and textural properties were analyzed using Dough and Gluten Extensibility Rig (A/KIE) which was attached to a 5 kg load cell. The pre-test speed, test speed, post-test speed, distance and trigger force used were 2 mm/s, 3 mm/s, 10 mm/s, 75 mm and 10 g respectively. Tensile strength (kPa) was calculated by dividing the maximum force at break by the

length and thickness of the film. The percentage change in film length was considered as elongation at break (%) of film

$$\text{Tensile strength} = \frac{Force}{Film\ thickness\ \ lengt\ h\ of\ Film}$$

Elongation (%) = [(Length after elongation – Actual length) × 100]/ Actual length

5.8.6. Water vapor permeability

Water vapor permeability (WVP) test of the film was carried out by following the modified method of ASTM standard. Water containing test cups (1.5 cm below the film) with silica gel will be taken and a plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion shall represent the steady state amount of water vapor transmission through the film will be calculated.

5.8.7. Mechanical test

Mechanical properties of the cellulose nano paper were investigated in terms of the tensile strength. Universal testing machine (Zwish et al., 2010) was employed for determination of tensile strength using test standard DIN EN ISO 527-1 for film and boards equipped with 100N load cell. The cellulose paper was cut into rectangular pieces of 5mm width and tensile stress –strain curve were recorded to a strain rate of 10% min and crosshead speed of 5mm/min. The thickness was measured to be 25µm and the tensile values were collected as the average value of three specimens.

5.8.8. Transmittance

Film transparency was determined by measuring the percent transmittance at 660 nm using a UV/VIS spectrophotometer (Mohanty et al., 2006).

5.8.9. XRD pattern

The powder X-ray diffraction patterns were measured using a Rigaku Miniflex instrument at ambient temperature using Cukλ radiation (λ=0.15418) over the 2Θ range of 5 and 50º with a scanning speed of1.2º/min. The crystallinity index was calculated using Segal method.

5.8.10. Fourier transform infrared spectroscopy (FT-IR)

IR spectra of CNFs were measured using KBr disk (Ultra thin pellets) method. The dry sample was Ground and blended with KBr in a ratio of sample/KBr 1:4. The blend was pressed to obtain a pellet and

introduced in the spectrometer (Nicolet Instrument 410 FTIR equipped with KBr optics and DTGS detertor, Themo Scientific, USA). Each spectrum was analyzed in the range of resolution from 500-4000cm^{-1} with a resolution of 4cm-1 and total of 64 scans were collected.

5.8.11. Biodegradability

In Biodegradability test, weight loss of the specimen was collected to be the extent to measure bio degradability. The microorganisms selected for this investigation were *pseudomonas aeruginosa* PAO1 (ATCC 15729), *pseudomonas aeruginosa* (ATCC 15692), *pseudomonas Putida* (3000 ATCC 10862). Bacterial cultures were stored in Iso-sensitest broth at 4ºC and were plated in Iso-sensitest agar for further investigation. The nutrient broth was made as the basal mineral media used for testing the biodegradability of LDPE consisted of the following elements (prepared in distilled water): 12.5 g $1^{-1}K_2HPO_4$; 3.8 $g1^{-1}KH_2PO_4$; 1.0 g $1^{-1}(NH_4)_2\ SO_4$; 0.1 g 1^{-1} $MgSO_4$. $7H_2O$ and 5 ml trace element solution which contained each of the following elements (prepared in distilled water): 0.232 g 1^{-1} $ZnSO_4$. $7H_2O$; 0.116 $g1^{-1}$ $FeSO_4$ $(NH4)_2$ $SO_4.6H_2$; 0.096 g $_1{}^{-1}CoSO_4.7H_2O$; $0.022g1^{-1}(NH4)6Mo7O_2.4H_2O$; $8.0mg1^{-1}CuSO_4.5H_2O$.

The cellulose and Nanocellulose after exposure to all four bacterial suspension were taken and washed thoroughly with 2% SDS for 4h. The strips were then dried at 60°C over night.

Percentage weight loss was determined using the following formula

$$\text{Weight loss} = \frac{Initial\ \ wt - final\ \ wt}{final\ \ wt} \times 100$$

5.9. Assessment of shelf life study of grape using cellulose and nano cellulose films:

Firstly, we took two fresh red grapes from market. The two grapes were then wrapped by two different films. i.e., (i) cellulose film; (ii) nanocellulose film. The wrapped red grapes were then placed inside the incubator at a temperature 35°C for 9 days.

Results and discussion

Cellulose Purity and Density Determination:

Purity of cellulose content in culinary banana was found to be 92% in method – I and 89% in method – II and density was found to be 0.23% in method – I and 0.22% in method – II respectively.

Table 5.1 Cellulose purity and density determination

Percent purity of cellulose	Purity (%)	Density (g/ml)
METHOD - I	92	0.23
METHOD - II	89	0.22

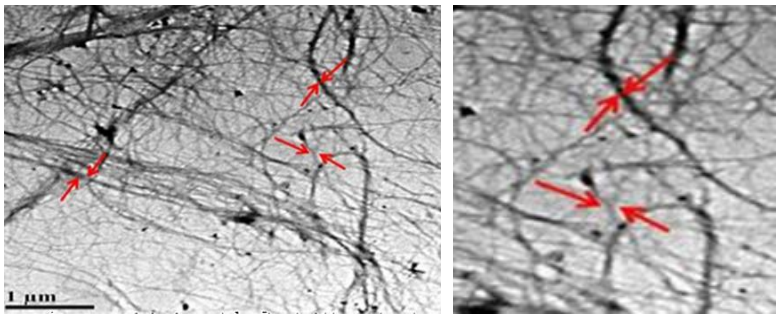
Characterization of cellulose and nanocellulose

1. **TEM (Transmission electron microscope)**

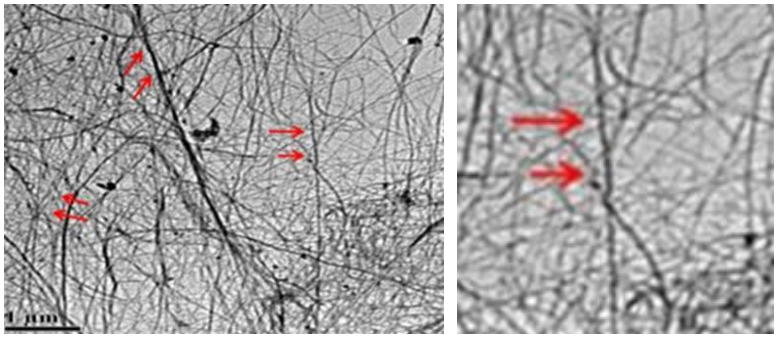
TEM image of cellulose nanofibers after 30 min of high intensity ultrasonication at 800w confirmed the formation of the nanofiber. It could be see that the chemical treatment eradicated the entire amorphous compound and only needle like cellulose nanofibers were present. Application of high intensity sonication gave better result in TEM along with chemical treatment. Tibolla, et al (2011) isolated cellulose nano cellulose from banana flower and from the TEM analysis showed that the chemical treatment gives better result than from the enzymatic treatment applied to the sample. This method of using ultra sonication in place of enzymatic is very simple and also very cost effective as found from the other word done on the nano cellulose this method has more reproducibility and is a very reliable technique.

Table 5.2 Particle size distribution of cellulose nanofibers (CNFs) observed by TEM

Sample	**Diameter, D(nm)**	**Length, L(nm)**	**Aspect ratio(L/D)**
Cellulose	35 ± 9.5	540.67 ± 3.4	16.14 ± 6.37
Nanocellulose	29 ± 6.6	364.9 ± 5.3	14.34 ± 6.02



(A) Cellulose fiber tube structure (right) and magnify cellulose fiber tube (left)

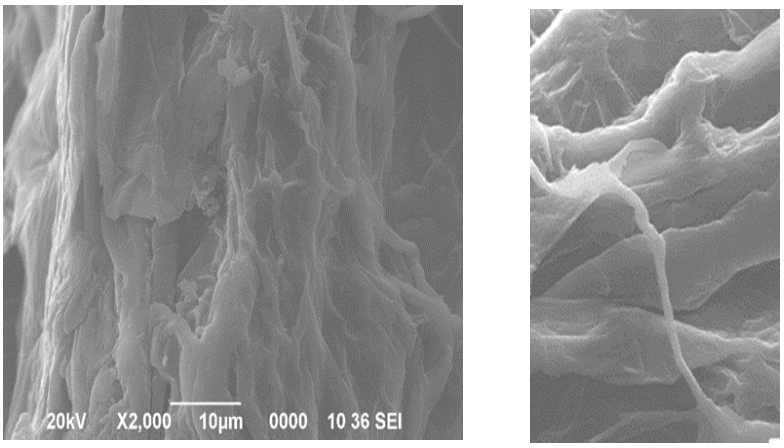


(B) Nanocellulose fiber tube structure (right) and magnify nanocellulose fiber tube (left)

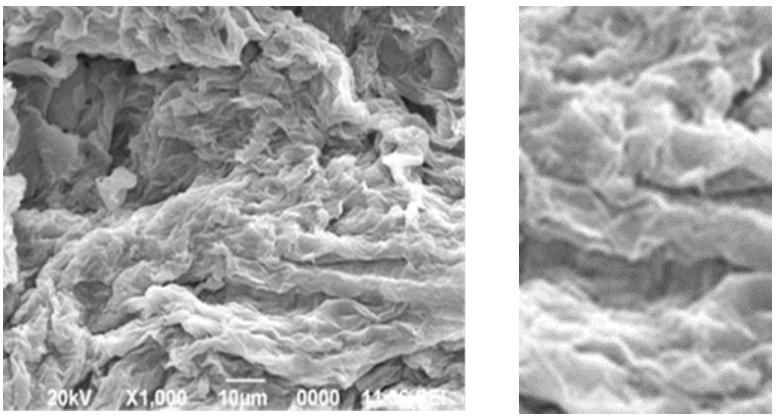
Fig. 5.4 TEM micrograph: (A) Cellulose fibers; (B) Nanocellulose fibers

2. SEM (Scanning Electron Micrograph)

The evaluation in the change of the structure of cellulose nanofiber was evaluated after subjecting to chemical and ultrasonic treatment. The scanning electron Micrograph shown below in Fig. 5.5 illustrates the structural morphology of cellulose and nanocellulose film. The microstructure of culinary banana flower showed irregular structure with presence of some starch granules. The morphology implies that the bleaching treatment employed to the sample was successful to remove the protein, mineral, lignin and hemi cellulose. To further shorter the chain of cellulose, ultrasonication was employed which was also successful. We cannot observe any nano-cellulose tubes in Fig. A; but tailoring by ultrasonication, we can see the nano cellulose tube in Fig. B. The ultrasonic treatment gives to tailor and reduce the size of nanofibers illustrated a visible fragmentation and tailoring of the nanofiber with respect to treatment given which has been confirmed by TEM analyses.



(A) Cellulose fiber tube structure (right) and magnify cellulose fiber tube (left)



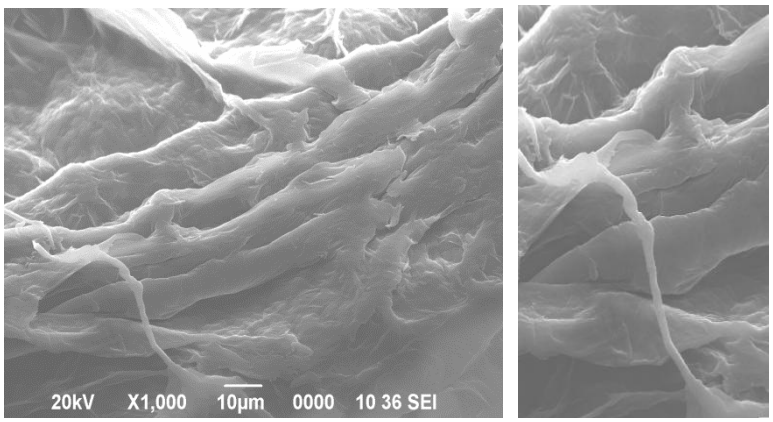
(B) Nanocellulose fiber tube structure (right) and magnify nanocellulose fiber tube (left)

Fig. 5.5 SEM micrographs

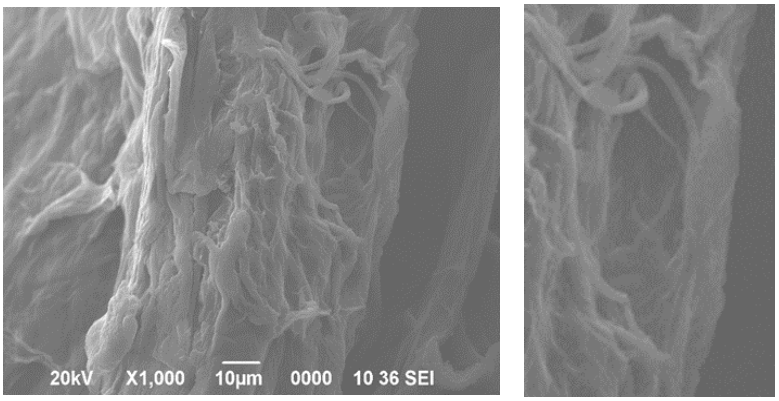
Characterization of cellulose and cellulose nanocellulose film

1. **SEM**

The scanning electron micrographs in Fig. 5.6 (A) and (B) illustrate the structural morphology of cellulose film and nanocellulose film. The chemical treatment employed for isolation of CNF was extremely effective and amorphours like lignin, pectin, hemicelluloses was adequately removed. The chemical treatment employed for isolation of CNF was extremely effective and amorphours like lignin, pectin, hemicelluloses was adequately removed. The chemical treatment employed for isolation of CNF was extremely effective and amorphours like lignin, pectin, hemicelluloses was adequately removed. Nanotubes are usually tube shaped hollow material with lesser in diameter than nanofibers.



(A) Cellulose fiber tube structure (right) and magnify cellulose fiber tube (left)



B) Nanocellulose fiber tube structure (right) and magnify nanocellulose fiber tube (left)

Fig. 5.6 SEM micrographs: (A) Cellulose film; (B) Nanocellulose film

2. Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared spectra of the cellulose and nanocellulose film were recorded in between 400-4000 cm^{-1} which helped to identify the function group present in the sample. A broad peak is observed at the spectral range between 3448cm^{-1} which represent the OH stretching which has resulted from the vibration of intermolecular hydrogen bond hydroxyl group. Same peak can be noted in case of nanocellulose. Around 817 cm^{-1} and 863cm^{-1} a small spectral band peak can be observed for cellulose and nanocellulose respectively which represent the COC, CCO and CCH deformation and stretching. At 632cm^{-1} a peak can be observed which represent C-OH out-of plane bending. At 1065 and 1000 a peak can be observed for cellulose and nano cellulose respectively which represent C-C, C-OH, C-H ring and side group vibration. At 1367 cm^{-1} peak is observed in case of nanocellulose which represent in plane C-H bending. At 1646 cm^{-1} peak on both the sample is observed which represent C-H symmetrical stretching.

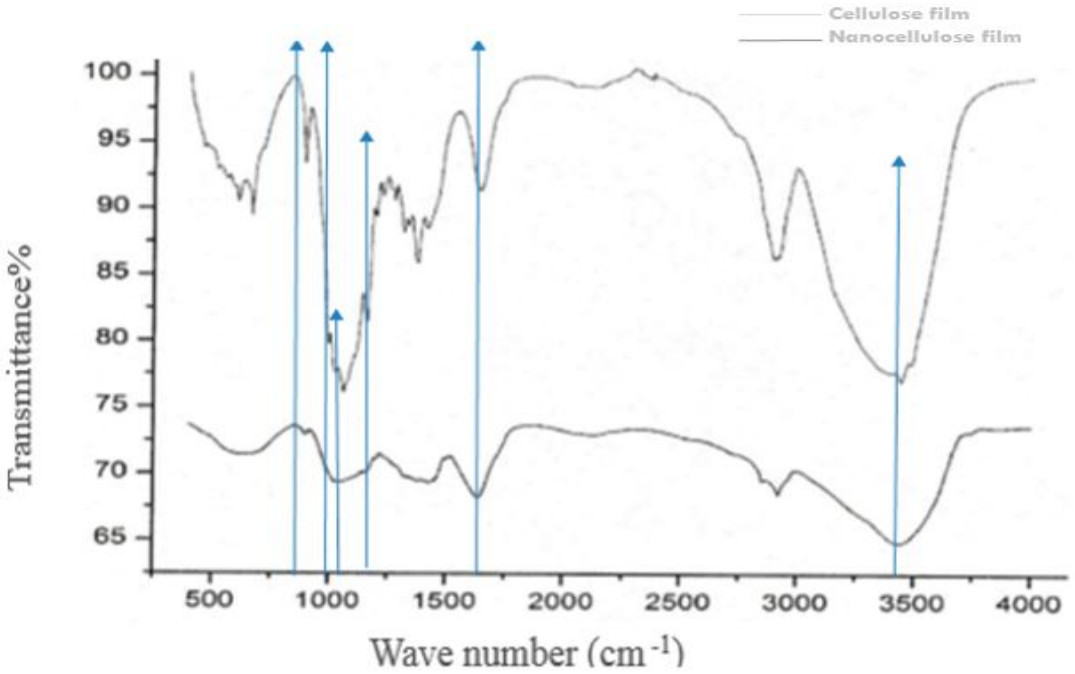


Fig. 5.7 FTIR spectra (cm^{-1}) for cellulose and nanocellulose film

3. X-ray diffraction (XRD)

The diffraction spectra was to evaluate the effect of combined chemical and ultrasonic treatment on the crystallinity and the crystal type of the extracted cellulose and the nano cellulose the exhibited the spectra of a typical cellulose I structure with peak at around 2Θ=16, 22 and 35 corresponding to the (110), (002) and (004) planes. The crystalline index (Ic) calculated for the cellulose and nanocellulose were found to be 42.30% and 67.76% respectively. The size of individual crystal of cellulose was smaller compared to nanocellulose may be attributed to the Ultrasonication treatment given for disintegration of fiber. On the hand, during acid hydrolysis treatment the hydronium ion penetrates into amorphous region of cellulose and release individual crystallities. The increase percent of crystallinity might be attributed to the ultrasonication of the cellulose which reduced the size of the cellulose and increased the crystalline cellulose the increase of crystallinity reported by Jihua et al. (2012) showed less crystallites in sugarcane bagasse.

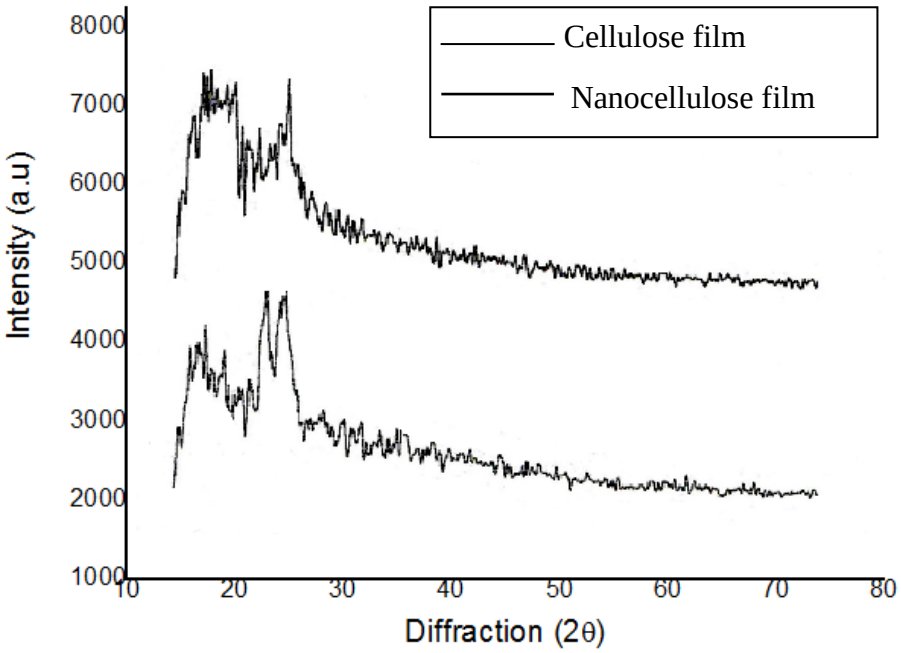


Fig. 5.8 XRD diffraction

4. Cellulose and Nanocellulose Film Thickness

The average thickness of the film ranged from 0.48 ± 0.01 to 0.40 ± 0.01mm. Thickness of the casting of solution into the petridish was 5 mm of the height. Casting the film forming solution in the petridish for drying played an important role in thickness of the film.

Table 5.3 Cellulose and Nanocellulose film thickness

Film	Average Thickness
Cellulose Film	0.48± 0.01
Nanocellulose Film	0.40±0.01

5. Swelling and Solubility of water

The swelling property of nanocellulose film was found from 20.22±0.16 to 22.60±2.78 %. This may due to the ultrasonication treatment at 800 W with increase in ultrasonication. Film solubility is an important property of biodegradability film because potential food application may require film good water insolubility to enhance product integrity and water resistance. Film solubility was found lowest in film treated with 30 min ultrasonicated treatment time and power of 800 W and found acceptable.

Table 5.4 Swelling and solubility of water

Film	Swelling %	Solubility %
Cellulose Film	9.20 ±1.77	52.72±1.33
Nanocellulose Film	14.11 ±1.62	60.46±4.28

6. Mechanical properties

In additional to the morphology, nano film derived from culinary banana flower exhibited good tensile property. The tensile property of the nanocellulose film was evaluated using uniaxial tensile text. The tensile exhibited by the nanocellulose was excellent. The maximum tensile strength was determined using the maximum force which is required to rupture the nanocellulose film was observed as 97.1 Mpa Sehaqui et al reported that cellulose nano film had a tensile strength at a range between 84Mpa.

7. Water Vapor Transmission Rate (WVTR)

The WVTR predicted the transmission of water vapor through a package and is considered to be a very important parameter. The WVTR of the CNP was measured by dish method. The films were kept in 63% RH at 25ºC. The WVTR achieved by the calculation following Rangana (1986) and the WVTR was found to be 145.2.0±g/m^2 day. Hence, it can be said that nanocellulose film has much better than cellulose film because of its high crystallinity. Zaopings et al. (2014) made nanocellulose film and its WVTR was observed to be 1317 g/m^2 day in 50% RH at 23ºC and increased to more than 2000g /m^2 day.

Table 5.5 Water vapor transmission rate (WVTR)

Film	Water vapor transmission rate (g/m^2)/24 h
Cellulose Film	27.70
Nanocellulose Film	26.56

8. Light Transmission and film transparency

The films were cut into rectangles (2×40) and placed in cuvette in a spectrometer cell. The barrier property was measured in 650 nm using UV-VIS spectrometer. According to Fang et al. (2002), the transparency was calculated and found to be 7.5±0.120% film transparency and light transmittance was found to 59.99% and for untreated cellulose film, it was found to be transparent and light transparency was found to be 23.37±0.43 and light transmission was found to be 29.82%. Savadekar et al. (2012) reported 62% light transmittance in 650 nm in CNF.

9. Differential Scanning Calorimetry

The onset temperature, peak temperature and melting point (Tm) of the two different films were measured using DSC. Samples weight ranges of 4.8–7.5 mg was taken both cellulose and nanocellulose film. Fig. 1 and Fig 2 showed the differential scanning calorimetry. The maximum point in the first endothermic peak is related to melting point. The melting point of nanocellulose film is highest (127.6°C) which shows that the film treated at high ultrasound treatment time and power (30 min and 800 W) is more resistance to temperature.

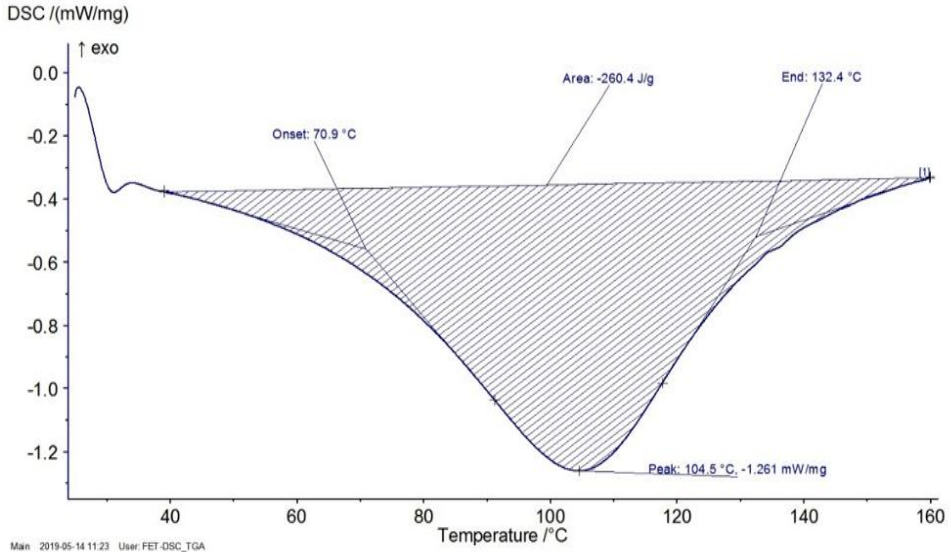


Fig. A: Cellulose film

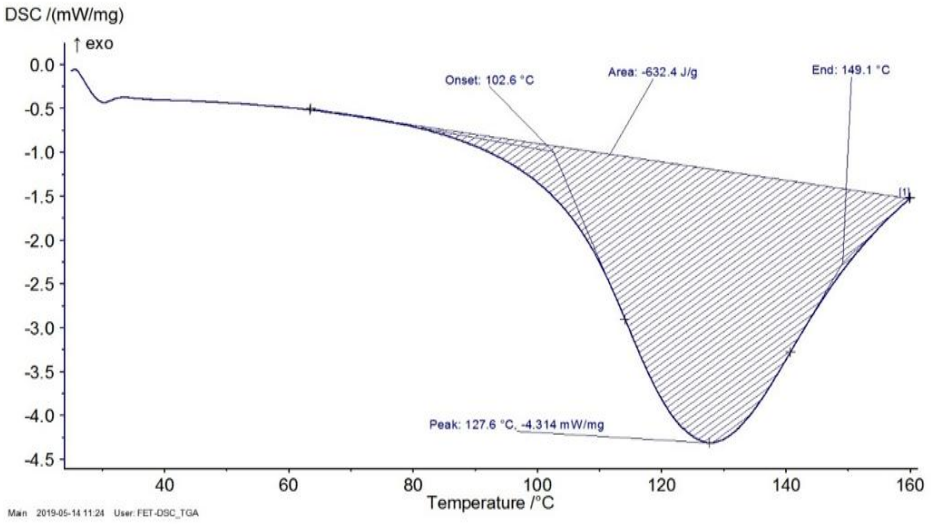


Fig. B: Nanocellulose film

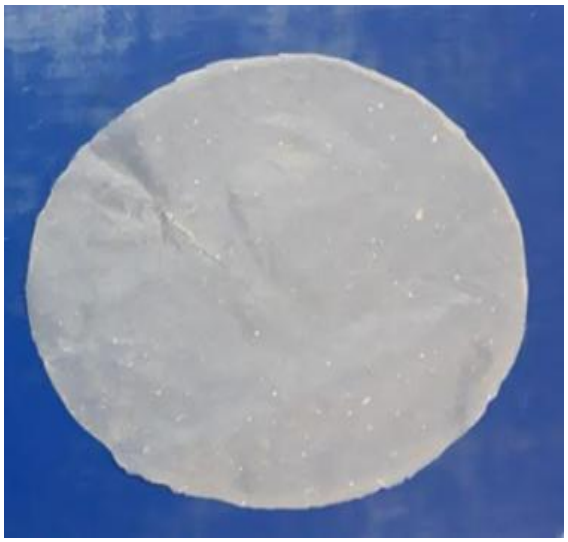
Fig. 5.7: DSC for (A) Cellulose film; (B) Nanocellulose film

10. Biodegradability

The sample along with the broth was kept at room temperature for 120 hours after the time period was completed. The sample was filtered and the cellulose nano film was collected and oven dried at 60ºC overnight and then weighed. The weight loss of the sample was found to be 23%. Hence, we can conclude that the cellulose nano film is bio degradable.

11. Shelf life study of black grape using newly developed nanocellulose films (Food preservation application):

In order to evaluate the potential application of the prepared nanocellulose composite films in food packaging, the shelf life of black grape was studied with plastic wrap as control. Pictures were taken initially after 9 days of storage at 37 °C as shown in Fig. 5.6. When wrapped with cellulose film, the black grapes showed obvious mildew appearance containing several moldy spots and sticky juice leaked to the surface. (Fig.5.8 A). The black grape wrapped by nanocellulose composite film (Fig.5.9 B) was still fresh without putridity. The results showed that the prepared nanocellulose composite film was a promising food packaging material which could protect food from microbial infection and extend its shelf life.



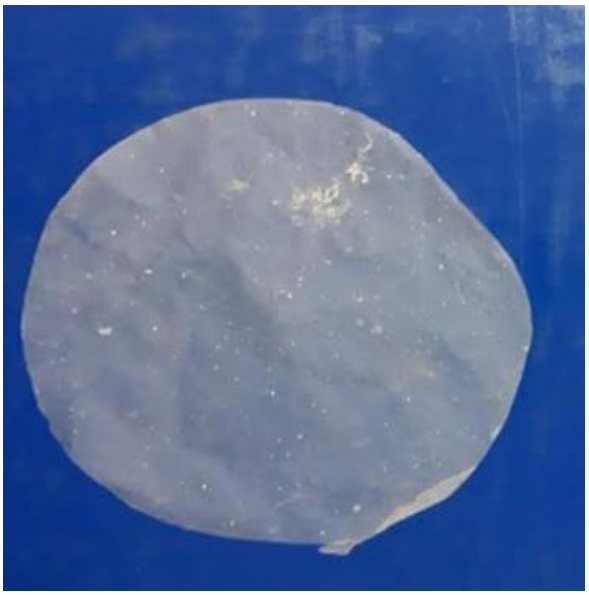
(A) Cellulose film

(B) Fresh black grapes

(C) Grape wrapped with cellulose film

(D) Appearance containing several moldy spots and sticky

Fig. 5.8 Shelf life study on cellulose packaging film: (A) Cellulose; (B) Fresh black grape; (C) Grape wrapped with cellulose film; (D) Appearance containing several moldy spots and sticky



(A) Nanocellulose film

(B) Fresh Black Grapes

(C) Grape wrapped with nanocellulose film

(D) Appearance still fresh without putridity

Fig. 5.9 Shelf life study on nanocellulose packaging film: (A) Nanocellulose film; (B) Fresh black grape; (C) Grape wrapped with nanocellulose film; (D) Appearance still fresh without putridity

Objective 6: Developed product to be tested among the local population for acceptance

Materials and methods

Acceptability analysis of the developed product among the local population

The requirements for panel membership was: i) good health; ii) average sensitivity; iii) high degree of personal integrity; iv) intellectual curiosity and interest in sensory evaluation work; v) ability to

concentrate and learn; and vi) availability and willingness to spend time in evaluation and submission to periodic tests for acuity and consistency. Candidates possessing these qualities were indexed with details of age, sex, specific likes and dislikes, availability, etc. The candidates were then put through basic qualifying tests of odour and taste recognition and thresholds, and their performance was recorded in the index cards. Judging was done in individuals booths. This assures independent judgement and no communication between panel members was allowed except for consultation with the panel leader on any point of doubt. The sensory testing was done during 10.00 am to 12 noon and 3 to 5 pm. It was preferable that panel members do not smoke or chew pan or supari for at least half an hour prior to the test. Judgement was done quickly, but not hurriedly. Odour observations by sniffing were done before tasting. While panel members were allowed retesting, prolonged testing was not conductive to reliable judgements. Tasters were either allowed to swallow or spit out the samples after testing. Rinsing between samples to remove flavor was recommended. In every evaluation, the procedure followed was adhered to by all and for all samples. These tests were performed by 30 panelists out of which 12 were females and 15 were males. Each panelist was served with each different sample. The overall acceptability based on color, texture, taste and flavour was evaluated on a 9-point unstructured hedonic scale ranging from 9 (Extremely like), 8 (Like very much), 7 (Like moderately), 6 (Like slightly), 5 (Neither like nor dislike), 4 (Dislike slightly), 3 (Dislike moderately), 2 (Dislike very much), 1 (Dislike extremely). For the preference ranking test, the panelists was asked to choose the most and least preferred sample based on their overall impressions (Oliveira et al., 2012).

Results and discussion

Three different cookies with 1%, 3%, and 5% of dietary fibre concentrate extracted from culinary banana flower (*Musa* ABB) and the control developed were taken for overall acceptability analysis. Developed products were tested among the local population for acceptance. Acceptability scores with five different attributes viz., color, texture, taste, flavor and overall acceptability are shown in Table 6.1.

Table 6.1 Acceptability score of cookies

Parameters	Control	1% DF	3% DF	5% DF
Color	8.01±0.27	8.54±0.32	7.14±0.12	7.31±0.13
Texture	7.86±0.39	7.15±0.49	7.46±0.89	7.36±0.21
Taste	8.54±0.18	7.27±0.24	7.37±0.15	7.82±0.24
Flavor	7.80±0.23	7.72±0.37	8.34±0.47	8.11±0.39
Overall acceptibility	8.41±0.16	7.65±0.23	7.83±0.69	7.76±0.57

From the above Table 6.1, we can observe that the overall acceptability of the developed products with different percentage of dietary fibre concentrate. The overall acceptability of the developed products was found not to be highly different from each other. The product with 3% dietary fibre concentrate with 7.83±0.69 points was reported to have the highest overall acceptability, followed by 5% dietary fibre concentrate (7.76±0.57 points) and 1% dietary fibre concentrate (7.65±0.23 points).

Dietary fibre concentrate (DF) also has significant impact on the attributes of bread, its starch digestibility and pGI. The increased incorporation of DF (2-4%) resulted in enhancement of stiffness, consistency and elasticity of dough as indicated by higher G′. The highest total DF was obtained with higher incorporation of DF (4% of flour mixture). Increasing DF level (2-4%) significantly decreased specific volume and produced firmer bread with high chewiness and less springiness as evident from texture analysis data. Therefore, incorporation of DF (2% of flour mixture) with 66% moisture level was chosen as it produced nutritionally balanced bread and not significantly different from control (higher specific volume, lower hardness, high springiness). Besides, it had a prominent acceptability attributes in terms of color, taste, texture and appearance.

SUMMARY AND CONCLUSION

Banana harvest includes waste in large amount every year. Culinary banana flower are fractions which are discarded. There has been limited works till now on the culinary banana flower. In spite of the fact that banana flower is very rich in fiber and cellulose it is not utilized to its full extent. Also, there is great need to reduce the amount of waste generated and its potential use in food related applications. The present study revealed that both outer and inner bracts are potential sources of DF along with other nutrients and phytochemicals. The outer and inner bracts contained higher total DF of 61.00% and 66.18%. Both outer and inner bracts contained significant amount of bound polyphenols and could be considered as antioxidant rich DF. The total polyphenol content of outer and inner bracts was 7.56 and 9.44 mg phenols/g dry sample, respectively. Moreover, better functional properties (e.g. WHC, OHC, WSC and GDRI) evidenced that outer and inner bracts are superior to cellulose and many other DF rich sources. The WSC of outer and inner bracts was 15.51 and 8.9 ml/g, respectively and was found significantly higher when compared to cellulose (4.5 ml/g). The retardation of glucose diffusion at different points of time (20, 40 and 60 min) for inner bract, outer bract, cellulose (reference) and control was taken. The dialysate glucose content of control increased gradually (0.16 to 0.38 mg) with time (20 to 60 min). However, the dialysate glucose content of outer and inner bracts increased in a lower rate as compared to control and increased from 0.1 mg to 0.14 mg and 0.09 mg to 0.15 mg, respectively with

time 20 to 40 min. All-inclusive results evinced that the outer bracts along with the inner bracts constitute a good source of DF associated with polyphenols and its excellent functional properties rendered it more suitable source of DF. The extracted dietary fibre concentrate extracted from culinary banana flower (*Musa* ABB) showed overall nontoxic properties: negligible effect on cell membrane stability, non-haemolytic, HPBMs were viable at its different concentrations. The results indicated that the extracted dietary fibre concentrate were nontoxic towards peripheral blood mononuclear cell (PBMC). Also, with increase in the concentration of dietary fibre extract, %viability of the two human colon cell lines i.e. HT-29 cells were found to be decreased and possessed anticancer property as observed in HT-29 cancerous cell line. The dietary fibres were incorporated into cookies and the proximate compositions of cookies were determined. Also, some physical parameters like thickness, diameter and spread ratio were determined. The incorporation of DF gradually increased the antioxidant properties in cookies with increasing concentration of DF (1% DF, 3% DF and 5%DF). Previous reports suggested that incorporation with dietary fibre improve the antioxidant properties of cookies. Radical scavenging potential of the cookies were measured in terms of DPPH radical scavenging activity. The incorporation of DF progressively increased the radical scavenging activity of the cookies. This may be correlated with the increase in DF content of the cookies. Thus, incorporation of DF improves the health benefits by increasing antioxidant activity and its dietary fibre content. The color properties were also checked and the changes occurred with the increase of DF were also noticed. Dietary fibre concentrate (DF) also has significant impact on the attributes of bread, its starch digestibility and pGI. The increased incorporation of DF (2-4%) resulted in enhancement of stiffness, consistency and elasticity of dough as indicated by higher G′. The highest total DF was obtained with higher incorporation of DF (4% of flour mixture). Increasing DF level (2-4%) significantly decreased specific volume and produced firmer bread with high chewiness and less springiness as evident from texture analysis data. Therefore, incorporation of DF (2% of flour mixture) with 66% moisture level was chosen as it produced nutritionally balanced bread and not significantly different from control (higher specific volume, lower hardness, high springiness). Besides, it had a prominent acceptability attributes in terms of color, taste, texture and appearance. No studies have been done on utilizing and modifying cellulose from culinary banana into nanocellulose. In few studies conducted on micro fibrillated cellulose and nanocellulose, it has been found that the paper made from it are very hard and can withstand high tension force along with the much needed feature of being biodegradable. Based on the above mentioned issues and new features of this nanocellulose, this present work has been carried out to show the potential utilization of nanocellulose formed by combined chemical and ultrasonic treatment of cellulose banana flower. This work has been done with an aim to decrease waste material and to make a packaging material with high barrier property and tensile strength, which is also biodegradable. Summing up the most significant contribution accomplished in this work, a

banana harvest waste was utilized i.e. culinary banana flower. The proximate composition of the culinary banana flower powder was investigated and a high amount of moisture in wet basis i.e. approximately 91% of banana flower was obtained. After removal of moisture the next major constituents found was fiber which was around 17.2% dry basis. The cellulose content was found to be 34%. Extraction of cellulose from banana flower was performed and two methods were employed to extract the cellulose from banana flower as it was found that method-I gave more percent of purity of cellulose. The cellulose was further ultrasonicated to form nanocellulose at a power level of 800 W. Characterization of cellulose nano film was done and the film was found to have good tensile strength greater than cellulose film. The nanocellulose film had a very good water vapour barrier property and found to be highly crystalline,which is a good property. The thickness of cellulose and nanocellulose was found to be 0.48± 0.01 and 0.40± 0.01 respectively, which displayed nanocellulose to be more thicker than cellulose. The swelling property of nanocellulose film ranges from 20.22±0.16 % to 22.60±2.78 %. This may be due to the high intensity ultrasonication treatment at 800 W. Differential Scanning Calorimetry showed the onset temperature, peak temperature and melting point (Tm) of the two different films. The melting point of nanocellulose film is observed to be highest (127.6°C) which shows that the film treated at high ultrasound treatment with time and power of 30 min and 800 W is more resistant to temperature. The transparency was calculated and found to be 7.2±0.114% nanocellulose film and light transmittance was found to be 68.73% and film transparency for cellulose film was found to be 22.45± 0.43 and light transmission was found to be 31.28%. Shelf life study of red grapes using newly developed nanocellulose films as food preservation application. The results of the present investigation has the credentials to support that both the outer and inner bracts of culinary banana flower could be commercially exploited for extraction of DF coupled with high antioxidant activity and has the potential as functional food to promote and maintain human health. Also, the red grapes wrapped with nanocellulose composite film were still fresh without putridity and the results showed that the prepared nanocellulose composite film was a promising food packaging material which could protect food from microbial infection and extend its shelf life.

SIGNIFICANT ACHIEVEMENTS

- Three different products enriched with dietary fibre concentrate and biopolymer based cellulose and nanocellulose from culinary banana flower were developed:
  - i) Cookies enriched with dietary fibre concentrate from culinary banana flower
  - ii) Bread fortified with dietary fibre concentrate from culinary banana flower
  - iii) Biofilms with biopolymer based cellulose and nanocellulose from culinary banana flower

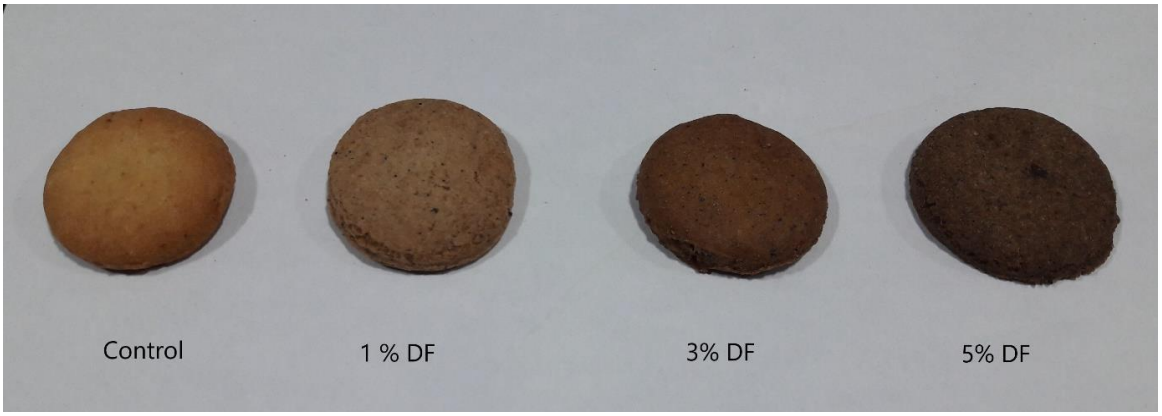


Fig.(i) Cookies with different concentrations of dietary fibre

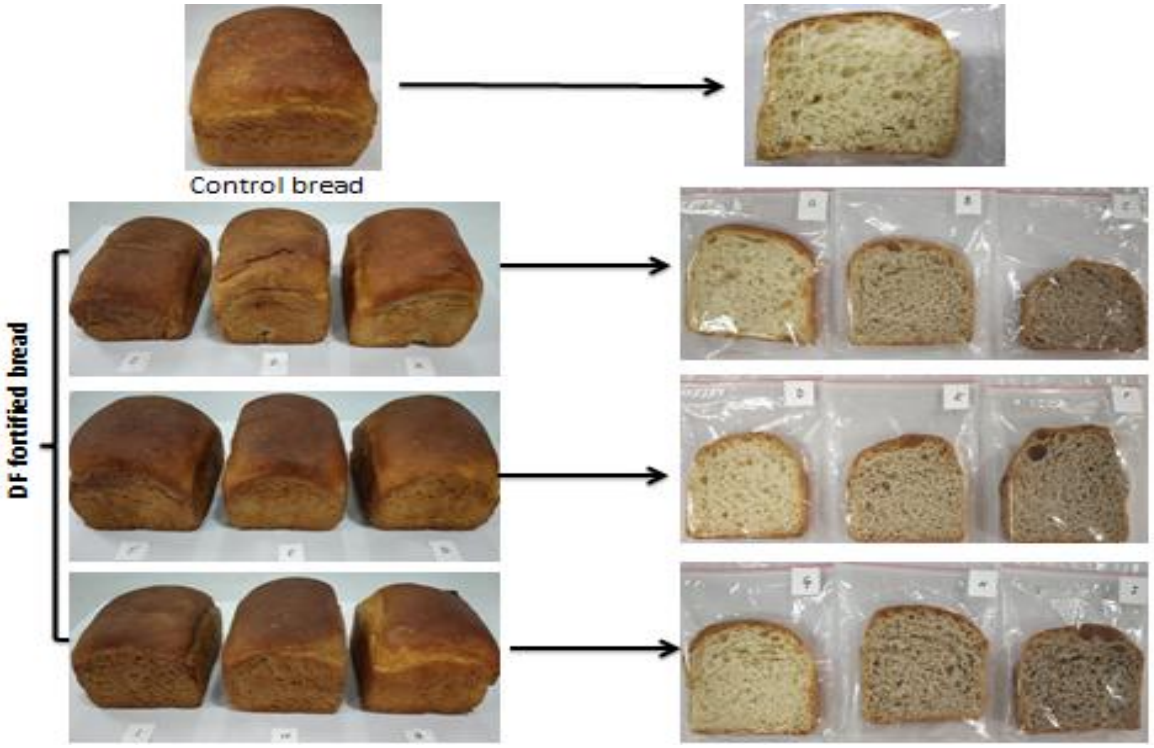
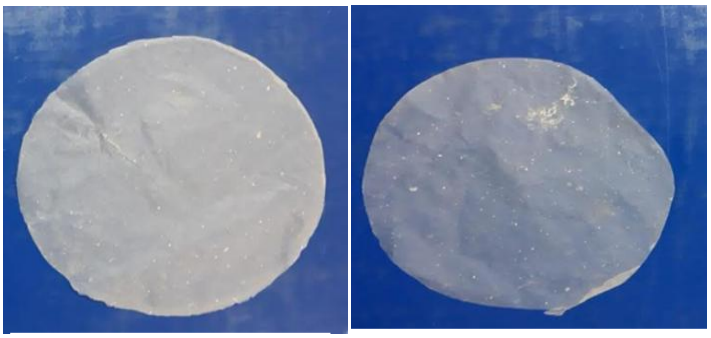


Fig.(ii) DF fortified breads along with the control bread



(a) Cellulose film (b) Nanocellulose film

Fig.(iii) Biopolymer based films: (a) Cellulose film; (b) Nanocellulose film

- Publications

1. Banana flower: A potential source of functional ingredients and its health beneficial effects. In: Applied Food Science and Engineering with Industrial Applications, International Apple Academic Press, Inc. (CRC Press, Taylor and Francis Group). (Book Chapter)

- Conference attended:

  Presented poster on topic "Bread fortified with dietary fibre extracted from culinary banana flower: its quality attributes and *in vitro* starch digestibility", IFCoN2018, organised by AFSTI, December12-15, 2018, CSIR-CFTRI, Mysore, India.

  Presented poster on topic "Bread fortified with anthocyanin-rich dietary fibre extracted from culinary banana bract (Page No. 60)". In: Souvenir, 27th Indian Convention of Food Scientists and Technologists (ICFoST)30th January -1st February 2020, Tezpur University, Assam, Organized by AFSTI, CFTRI, Tezpur University and DRDO, India.

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Name and Signature of
Principal Investigator
Date:

AUDITED UTILIZATION CERTIFICATE

FOR THE FINANCIAL YEAR 2019-2020 (w.e.f. 01.04.2019 to 31.03.2020)

1.	Title of the Project/Scheme		Value addition of banana flower using green technologies to develop combat food
2.	Name of the institution		Tezpur University
3.	Principal Investigator		Professor Sankar Chandra Deka
4.	DRDO Letter No. and date of sanctioning the project Date of Start of the Project		O/o DG (TM)/81/48222/LSRB-312/FSH-ABB/2017, dated 26.02.2018 26.02.2018
5.	Head of account as given in the original sanction letter		Major Head-2080 (Defence Services) R&D Minor Head-004 R&D
6.	Amount brought forward from the previous financial year quoting DRDO letter No. & date in which the authority to carry forward the said amount was given.		Rs. 93,689.00
7.	Amount received during the financial year (Please give no. and date of DRDO sanction letter for the amount)		Rs. 6,68,968.00 No. O/o DG (TM)/81/48222/LSRB-312/FSH-ABB/2019, dated 26.02.2019
8.	Amount of interest accrued, if any, from the grants		Rs.3133.00 (DD No.534609 Dated 10.07.2020)
9.	Total amount that was available for expenditure (excluding commitments) during the financial year (SL. No. 6+7+8)		Rs. 7,62,657.00
10.	Actual expenditure (excluding commitments) incurred during the financial year (up to 25.02.2020)		Rs. 6,58,220.00
11.	Balance amount available at the end of the financial year.		Rs. 1,04,437.00
12.	Unspent balance refunded, if any (Please give details of Cheque No. etc.)		DD No.534608 Dated 10.07.2020
13.	Amount allowed to be carried forward to the next financial year		-

AUDITED UTILIZATION CERTIFICATE

FY 2019-2020 (w.e.f. 01.04.2019 to 31.03.2020)

Certified that sum of **Rs. 6,68,968.00** was sanctioned as grants-in-aid during the year **2019-2020** in favour of **Tezpur University, Napaam, Tezpur, Assam** vide DRDO letter No No. O/o DG (TM)/81/48222/LSRB-312/FSH-ABB/2019, dated 26.02.2019 and **Rs. 6,68,968.00** released vide Letter No. No. O/o DG (TM)/81/48222/LSRB-312/FSH-ABB/2019, dated 26.02.2019, an amount of **Rs** 3133.00 accrued as interest (during FY 2019-20), a sum of **Rs. 6,58,220.00** has been utilized for the purpose for which it was sanctioned and that the balance of **Rs. 1,04,437.00** remaining unutilized at the end of the closing of the project has been returned to the CDA (R&D). The interest accrued during FY 2019-20 Rs. 3133.00 has also been returned separately to the PCDA (R&D), New Delhi.

Principal Investigator
Professor
Department of Food Engg. & Technology
Tezpur University
Napaam, Tezpur- 784028, Assam

Finance Officer
21/6/2020
Finance Officer
Tezpur University

Registrar, Tezpur University
(with official seal)
Registrar
Tezpur University

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised

1. Accounts audited by qualified Chartered Accountant appointed by this University as Internal Auditor.
2. The A.G. (Audit), Guwahati has already audited the account.
3. All the instrument(s), chemicals, consumables, etc. purchased from the grant are entered in the Log Book.

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS

21.07.2020

CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

Signature of Audit Authority
of Grantee Institution

AUDITED STATEMENT OF EXPENDITURE ACCOUNTS
FOR THE FINANCIAL YEAR 2019-2020 (01.04.2019 to 31.03.2020)

(a) Title of the Project: Value addition of banana flower using green technologies to develop combat food
(b) Sanctioned letter no. & date: O/o DG (TM)/81/48222/LSRB-312/FSH-ABB/2019, dated 26.02.2019
(c) Principal Investigator: Professor Sankar Chandra Deka
(d) Date of Start of the Project: 26.02.2018
(e) Total Sanctioned cost of the Project : in Rs. 22,73,557.00
(f) Grant received (Rs.) in I yr Rs. 15,60,900.00 II yr Rs. 6,68,968.00
(g) Total Grants received so far: Rs. 22,29,868.00

S. No.	Sanctioned Heads	Funds Sanctioned for the year Rs.	Funds released Rs.	Carried forward from Previous year Rs.	Funds available (iv+v) Rs.	Expenditure incurring during the FY 2019-20 Rs.	Balance (vi-vii) Rs.	Commitments Rs.	Total expenditure (vii+ix) Rs.
i	ii	iii	iv	v	vi	vii	viii	ix	x
(a)	Staff	3,24,000	3,24,000	61,123	-	2,71,552	-	-	2,71,552
(b)	Equipment	-	-	700	-	-	-	-	-
(c)	Operation & Maint.	-	-	-	-	-	-	-	-
(d)	Expendables	2,50,000	2,50,000	–812	-	2,50,000	-	-	2,50,000
(e)	Travel	55,000	55,000	32,700	-	53,816	-	-	53,816
(f)	Contingencies	18,870	18,870	–22	-	17,936	-	-	17,936
(g)	Research Consultant	-	-	-	-	-	-	-	-
(h)	Procured Service	-	-	-	-	-	-	-	-
	Institutional over head	64,787	64,787	-	-	64,916	-	-	64,916
	Interest earned, if any	-	-	-	-		-	-	
	TOTAL	**7,12,657**	7,12657	**93,689***	**7,62,657**	**6,58,220**	**1,04,437**	**-**	**6,58,220**
			Funds Actually Released by PCDA on 22.8.2019 is **Rs 6,68,968***		**(Addition of Rs 6,68,968*+ 93,689*)**				

Name and Signature of Principal Investigator
Date: 24.6.2020

Professor
Department of Food Engg. & Technology
Tezpur University
Napaam, Tezpur- 784028, Assam

Name and Signature of Finance Officer
Date: 25/6/2020

Finance Officer
Tezpur University

Signature of Registrar, Tezpur University
Date:

Registrar
Tezpur University

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS
21.07.2020
CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

A/c Payee Only

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जारी करने वाली शाखा
State Bank of India
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DEMAND DRAFT

Key: TABDAP
Sr. No: 159237

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मांगे जानेपर PCDA (R&D) ********** या उनके आदेश पर
ON DEMAND PAY OR ORDER

रुपये RUPEES One Lakh Four Thousand Four Hundred and Thirty Seven Only

अदा करें ₹ 104437.00

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Name of Applicant

Key: TABDAP Sr. No: 159237
TEZPUR UNIVERSITY R& D

AMOUNT BELOW 104438(1/6)

मूल्य प्राप्त / VALUE RECEIVED

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AUTHORISED SIGNATORY

शाखा प्रबंधक
BRANCH MANAGER

भारतीय स्टेट बैंक
STATE BANK OF INDIA
अदाकर्ता शाखा / DRAWEE BRANCH:NEW DELHI MAIN BRANCH
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रुपये RUPEES Three Thousand One Hundred and Thirty Three Only

अदा करें ₹ 3133.00

IOI 000471534609
Name of Applicant

Key: ROGBEQ Sr. No: 486370
TEZPUR UNIVERSITY R& D

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