CHAPTER 7

# *IN VITRO* PHYSICOCHEMICAL, PHYTOCHEMICAL AND FUNCTIONAL PROPERTIES OF FIBRE RICH FRACTIONS DERIVED FROM BY-PRODUCTS OF SIX DIFFERENT FRUITS

## 7.1. Introduction

Plants are naturally very rich in fibre commonly referred to as dietary fibre that has health promoting properties. Dietary fibre binds bile and water, helps in the bowel movement, increases the fecal mass, controls irritated bowel syndrome conditions as well as lowers blood glucose and cholesterol level. [1] The glucose and cholesterol lowering properties in turn helps in prevention and control of obesity, diabetes and coronary heart diseases. Dietary fibre enriched diet prevents colon cancer. This could be due to the production of short chain fatty acids in the large intestine as by-products of the dietary fibre that are fermented by the gut micro flora residing there. A healthy adult person is usually recommended to include 25-30 g fibre in the daily diet. [2] Fruits and vegetables are naturally rich in dietary fibres. Fruit and vegetable by-products like peel, pomace, seed and seed coats are the major sources of dietary fibre. In most cases, the dietary fibres derived from fruits and vegetable by-products contain bioactive compounds embedded in their matrix [3] and therefore both fibre and antioxidant could impart health benefits. The bioactive compounds, mainly phenolic acids and flavonoids are the secondary plant metabolites that have health promoting properties like antioxidant and radical quenching properties. The awareness of the health benefits of dietary fibre and other bioactive compounds in the diet has led to a rapid development of fibre and antioxidant enriched food products.[4] Fernandez-Gines et al. [5] reported that addition of citrus fibre with associated antioxidant bioactive compounds to meat products inhibited lipid oxidation and decreased residual nitrite levels. Similarly, when mango peel powder was fortified into macaroni, an increase in the dietary fibre, polyphenols and carotenoid content was reported. [6]

Thus, a need has arisen to identify and develop new dietary fibre sources to cater to the increased industry demand for developing newer and efficient functional foods. [7] Therefore, the present work intends to do a comparative study of the health promoting and

functional properties of the fibres obtained as by-products taken from six sources viz., pomace of carambola (*Averrhoa carambola* L.) and pineapple (*Ananas comosus* L._*Merr*), peels of watermelon *(Citrullus lanatus)*, Burmese grape (*Baccurea sapida* Muell. Arg.) and Khasi mandarin orange (*Citrus reticulate* Blanco.*)*, and blossom of seeded banana (*Musa balbisiana* Colla. *ABB*)

## 7.2. Materials and methods

### 7.2.1. Chemicals and reagents

All the chemicals and reagents used were of analytical grade and supplied by Merck India Ltd. The dietary fibre enzyme kit was supplied by Sigma Chemicals Ltd, India and the glucose assay kit was supplied by Coral Clinical Systems, Tulip group, India.

All the fibre sources were procured from the local market, Tezpur. The peels or outer rind from Khasi mandarin (KMPL), Burmese grape (BGPL) and watermelon (WMPL) were removed and chopped into $1cm^2$ size and dried. Pomace from pineapple (PNPM) and carambola (CMPM) was collected after the extraction of their juice using a household juicer (Philips). In case of banana blossom (BB), the outer hard layer of the flower was discarded and the whole blossom was shredded and dried. All the samples were dried at 50°C in a tray drier for 12 h. The dried samples were ground in a laboratory grain mill (Fritsch Pulverisette, Germany) to pass through 0.5 mm screen and stored at refrigerated conditions in airtight containers for further analysis.

### 7.2.2. Proximate composition

#### 7.2.2.1. *Moisture content*

The moisture content of the prepared fibre samples were determined by AOAC [8] method. Briefly, the fibre sample was taken in previously dried and weighed covered dishes. The sample was allowed to dry in a hot air oven (Jiotech, South Korea) at 105°C for 8 h till a constant weight was attained. The final weight of the dish containing the sample was measured both before and after drying and moisture content was calculated.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100 \qquad \textbf{Eq. 7.1}$$

Where, $W_1$ is weight of the sample with the dish before drying; $W_2$ is final weight of the sample with dish after drying.

### 7.2.2.2. *Crude protein content*

The crude protein content was determined by modified Micro-Kjeldahl method of AOAC [8] using a digestion and distillation system (KelPlus, Pelican Equipment, Chennai, India). Briefly, 250 mg of the fibre sample was weighed and then digested with 10 mL concentrated sulphuric acid in a digestion tube at 350°C till a bluish green colour appeared. The digested sample was then distilled using 40 mL of 40 M sodium hydroxide with 20% of 20 mL boric acid with 1-2 drops of methyl red indicator. During distillation the ammonium vapor produced is passed over the boric acid solution which results in change of its colour from pink to light yellow. After completion of the distillation process, the distillate was titrated against 0.1 N hydrochloric acid and the end point was noted when a light pink colour appeared. The nitrogen content of the sample was calculated. The crude protein was calculated by multiplying a factor of 6.25 with nitrogen content.

$$\text{Nitrogen content (\%, db)} = \frac{S - B \times normality\ of\ HCL \times 14 \times 100}{Weight\ of\ sample \times 1000} \qquad \textbf{Eq. 7.2}$$

Where, S is the titre value of sample and B is the titre value of blank

$$\text{Crude protein content (\%, db)} = Nitrogen\ content\ (\%) \times 6.25 \qquad \textbf{Eq. 7.3}$$

### 7.2.2.3. *Crude lipid content*

The crude lipid content was determined as per AOAC method [8] using a Soxhlet apparatus (Socs Plus, Pelican Equipment, India). Briefly, 2 g of sample was weighed and extracted with petroleum ether (60°C-80°C boiling range) for 2 h at 100°C and recovered for 2 h at 200°C in a pre-weighed glass jar. Once the recovery process was over, the glass jars were put into a hot air oven at 105°C to remove any trace of moisture, cooled in a desiccator and then the final weight was measured. The total fat content was calculated.

$$\text{Crude lipid content (\%, db)} = \frac{W_2 - W_1}{S} \times 100 \qquad \textbf{Eq. 7.4}$$

Where, $W_1$ is the initial weight of the glass jar; $W_2$ is the final weight of the glass jar; S is the weight of the sample.

### 7.2.2.4. *Ash content*

The ash content was determined by AOAC method. [8] Briefly, 5 g of sample was weighed into a pre-weighed sintered crucible ($W_1$), burnt on a hot plate and ashed at 625°C

for 6 h in a muffle furnace (Labtech). The ashed sample with the crucible was then cooled in a desiccator and the final weight of the crucible ($W_2$) was measured and the total ash content was calculated.

$$\text{Ash content (\%)} = \frac{W_2 - W_1}{S} \times 100 \qquad \textbf{Eq. 7.5}$$

Where, $W_1$ is the initial weight of the empty crucible; $W_2$ is the final weight of the crucible after ashing; S is the weight of the sample.

### 7.2.3. Preparation of the Fibre Rich Fraction

The fibre samples were first subjected to hexane treatment for removal of crude lipids and pigments. The samples were kept overnight in hexane in the ratio of 1:10 (sample: hexane) at room temperature and then filtered with a muslin cloth. The residue left was then dried and again treated twice with 80% hot (80°C) ethanol for 30 min and filtered. The residue was rinsed with 95% ethanol and then dried at 50°C in a tray drier. The final dried solids were then ground to 0.5 mm particle size in a grain miller. The yield of the obtained fibre powder was calculated on dry basis.

$$\text{Yield (\%)} = \frac{W_1}{W_2} \times 100 \qquad \textbf{Eq. 7.6}$$

Where, $W_1$ is the final weight of the powder and $W_2$ is the initial weight of the sample.

### 7.2.4. Colour and dietary fibre content

#### 7.2.4.1. *Colour determination*

The colour variation among the obtained fibre samples was determined in a Hunter colour lab (Hunter ColourLab UltrascanVis). The '*L*' value indicates degree of lightness. '*L*' value in the range between 0-50 indicates dark and 51-100 indicates light. Similarly, '*a*' means measure of red (positive values) and green colour (negative values); '*b*' measures the yellow (positive value) or blue (negative values) colours. [9]

#### 7.2.4.2. *Determination of the total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) content of the prepared fibre samples*

The dietary fibre content was determined by enzymatic gravimetric method. [8] The fibre sample (1 g) was homogenized in 40 mL of 0.05 M 2-(*N*-morpholino) ethanesulfonic

acid (MES) buffer (pH 8.2), followed by the addition of 50 μL α-amylase (product code A 3306, termamyl, Sigma) and was incubated at 95°C for 15 min with occasional stirring. The contents was cooled to 60°C and rinsed with 10 mL of water. Then 100 μL of protease (product code P 3910, Sigma) was added and incubated at 60° C for 30 min. To this 5mL 0.561 M hydrochloric acid was added and stirred. The pH was adjusted to 4-4.7 at 60°C by adding 1 M hydrochloric acid. Lastly, 300 μL of amyloglucosidase (product code A 9913, Sigma) was added and incubated for another 30 min at 60°C with constant stirring.

***Insoluble dietary fibre determination***

The digested sample was filtered by vacuum filtration through a dried and pre-weighed crucible containing 0.5 g of celite. (Drying and weighing of crucible was done by adding 0.5 g of celite into the crucible and washing it with 20 mL of 95% ethanol and 20 mL acetone. It was then dried in oven at 105°C for 30 min and weighed). The residue was washed twice with 10 mL of hot water (70°C). The combined filtrate and water washings were then transferred into another beaker and kept for soluble dietary fibre determination. The washed insoluble residue was then washed twice with 15 mL portions each of 78% ethanol, 95% ethanol and acetone. The crucible was dried at 105°C overnight and the final weight was recorded.

$$\text{IDF} = \frac{W_1 + W_2/2 - P - A - B}{M_1 + M_2/2} \times 100 \qquad \textbf{Eq. 7.7}$$

Where, $W_1$ and $W_2$ are the residue weights of the duplicate test samples; $M_1$and $M_2$ are the weight of the test samples in duplicate; P is the weight of protein; A is the weight of ash content; and B is the test blank.

***Soluble dietary fibre determination***

The combined filtrate was measured first for total volume and then added four volumes of 95% ethanol preheated to 60°C. The filtrate and ethanol mixture was then allowed to precipitate at room temperature for 1 h. The precipitate was collected by vacuum filtration through a dried and weighed crucible containing 0.5 g of celite. (Drying and weighing of crucible was done by adding 0.5 g of celite into the crucible and washing it with 20 mL of 95% ethanol and 20 mL acetone. It was then dried in oven at 105° C for 30 min

and weighed). The crucible was dried at 105°C overnight and the final weight was recorded. Soluble dietary fibre (SDF) was calculated.

$$SDF = \frac{W_3 + W_4/2 - P - A - B}{M_1 + M_2/2} \times 100 \qquad \textbf{Eq. 7.8}$$

Where, $W_3$ and $W_4$ are the precipitate weights of the duplicate test samples; $M_1$and $M_2$ are the weights of the test samples in duplicate; P is the weight of protein; A is the weight of ash content; and B is the test blank.

***Total dietary fibre calculation***

$$TDF\ (\%) = IDF\ (\%) + SDF\ (\%) \qquad \textbf{Eq. 7.9}$$

Where, TDF is total dietary fibre, IDF is insoluble dietary fibre and SDF is soluble dietary fibre.

### 7.2.5. Physicochemical Properties

#### 7.2.5.1. *Moisture content*

It was determined by the method as mentioned **7.2.2.1.**

#### 7.2.5.2. *Bulk density*

The bulk density was calculated by weighing 1g of sample powder into a graduated 10 mL cylinder and measuring the volume occupied by the sample. The results are expressed as g/mL.

$$\text{Bulk density (g/mL)} = \frac{Weight\ of\ sample}{Volume\ occupied} \qquad \textbf{Eq. 7.10}$$

#### 7.2.5.3. *Water holding capacity*

Water holding capacity of the fibre samples was determined according to Robertson et al. [10] Briefly, 100 mg of sample was added to 10 mL distilled water and stirred overnight. Then the mixture was centrifuged in 50 mL graduated tube at 14,000 x g for 20 min. Water holding capacity (WHC) was expressed as g of water held per g of sample. Cellulose (Himedia, India) was taken as control.

#### 7.2.5.4. *Swelling capacity*

It was determined by the method of Robertson et al. [10] using cellulose as a standard control. For the determination, 100 mg sample was mixed with 10 mL distilled water in a graduated cylinder and allowed for equilibration at room temperature for 18 h. The swelling

capacity was calculated as the volume occupied by the fibre sample divided by the original sample weight (mL/g).

#### 7.2.5.5. *Oil holding capacity*

Oil holding capacity (OHC) was determined according to Lin et al. [11] Briefly, in a 50 mL graduated centrifuged tube, 100 mg of fibre sample was mixed with 15 mL of olive oil. The prepared solution was homogenized for 30 s every 5 min for 30 min and then centrifuged for 25 min at 1600 x g. The supernatant obtained was carefully removed and final weight of the residue was noted. OHC was expressed as g of oil held per g of sample. Cellulose was taken as control.

### 7.2.6. Phytochemical content and antioxidant activity

#### 7.2.6.1. *Sample extraction*

The fibre samples were extracted in 80% acetone for 90 min at 20°C in a ratio of 1:10 (sample: solvent) in a shaking incubator (Sartorius). After the completion of the incubation period, the extraction mixture was then centrifuged (Hettich-Zentrifugen, Germany) at 3000 rpm for 15 min and the supernatant was collected and stored at -20°C until further analysis of phytochemical and antioxidant activity.

#### 7.2.6.2. *Determination of total phenolic content*

Total phenolic content in the sample extracts was assessed using the Folin–Ciocalteau assay [12] with slight modification. For the analysis, 20 µL each of extract, gallic acid standard and blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteau reagent, mixed well and within 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40°C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). The results were expressed in mgGAE/100g.

#### 7.2.6.3. *Determination of total flavonoid content*

The flavonoid content was determined by aluminium trichloride method. [13] Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1mL of 10% aluminum trichloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of deionised water. After incubation at room temperature for 40 min, the absorbance of reaction mixture was

measured at 415 nm against deionised water taken as blank in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). Results were expressed as quercetin equivalent (mgQE/100g) of sample.

**7.2.6.4.** ***Determination of ferric reducing antioxidant property (FRAP)***

FRAP activity of the samples was measured by the method of Benzie and Strain. [14] Briefly, a 40 µL an aliquot of sample extract was mixed with 3.0 mL of FRAP solution. The reaction mixture was incubated at 37°C for 4 min and the absorbance was determined at 593 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37°C and prepared freshly by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5 mL of 20mM ferric chloride and 25 mL of 0.3M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1-10 mM). FRAP values were expressed as µmol of ferrous equivalent Fe (II)/100g of sample.

**7.2.6.5.** ***Determination of DPPH Activity***

Radical scavenging activity of the sample extracts was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. [15] For the experiment, 100 µL of extracts were added to 1.4 mL of 10-4 M DPPH radical methanolic solution. The absorbance at 517 nm was measured at 30 min against blank (100 µL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity.

Radical scavenging acitivity (%) = [(Ao-As)/ Ao] × 100 **Eq. 7.11**

Where, Ao is absorbance of control blank, and As is absorbance of sample extract.

**7.2.6.6.** ***Determination of Metal Chelation Capacity***

Metal chelating capacity was determined based on the method of Dinis et al. [16] For the estimation, 1.0 mL of 0.125 mM ferrous sulphate, and 1.0 mL of 0.3125 mM ferrozine were mixed with 0.2 mL sample. The mixture was allowed to equilibrate for 10 min at room temperature and the absorbance at 562 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) was recorded. The control contained all the reaction reagents except the extract. Decreased absorbance of the reaction mixture indicated increased activity.

Chelation activity [%] = [(Ao-As)/ Ao] ×100 **Eq. 7.12**

Where, Ao is absorbance of control blank, and As is absorbance of sample extract.

### 7.2.6.7. *RP-HPLC study of the polyphenols*

***Sample extraction***

The methanolic sample extract was prepared by extracting the fibre samples in 80% methanol acidified with 1% concentrated hydrochloric acid and dried under vacuum, then redissolved in 1 mL methanol and filtered using a 0.22 μm nylon filter (Himedia, India).

RP-HPLC (Waters system) gradient elution method was used to identify the major phenolic acid composition of the fibre samples. Symmetry 300$^{TM}$ $C_{18}$ (5 μm, 4.6 X 250 mm) column with a binary pump (Waters, 1525) and a UV-VIS detector (Waters, 2489) was used. Mobile phases used were acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method: 80% A (0-8 min), 65% A (9-12 min), 45% A (13-16 min), 30% A (17-20 min), 20% A (21-30 min), 10% of A (31-34 min) and then washing of the column with 65% A (35-39 min) and lastly, 80% A (40-45 min) was followed. Sample volume of 20 μL was used. The flow rate was maintained at 0.8mL/min and wavelengths used for UV-Vis detector were 254 nm and 325 nm. The standards used for comparison and identification were (±) catechin, caffeic acid, coumaric acid, gallic acid, syringic acid, chlorogenic acid, rutin hydrate, quercetin and ascorbic acid.

## 7.2.7. Functional properties

### 7.2.7.1. *Glucose adsorption capacity and amylase activity inhibition rate*

Glucose adsorption capacity was determined by the method given by Ou et al. [17] Briefly; 1 g of sample was mixed with 100 mL glucose solution (100mmol/L) and incubated at 37 °C for 6 h. Then, the sample was centrifuged at 4000 x g for 20 min. The glucose content of the supernatant was determined using glucose assay kit (Coral Clinical Systems, India). For the above study, cellulose was taken as the control to compare with that of the samples. The adsorbed glucose was calculated as the amount of glucose retained by the sample (mmoL/g of fibre).

Amylase activity inhibition rate was also determined by the method of Ou et al. [17] using cellulose as a control. One gram of sample and 4 mg of α- amylase was mixed in 40 mL potato starch solution (4%, w/v) and incubated at 37 °C for 30 min, and the final glucose

content in the mixture was measured using glucose assay kit (Coral Clinical Systems, India). The amylase inhibitory effect was expressed as rate of micromoles of glucose production per hour per gram of sample (μmoL/h/g).

#### 7.2.7.2. *Glucose diffusion rate and glucose diffusion reduction index (GDRI)*

Glucose diffusion and GDRI were determined according to the method of Ou et al. [17] with slight modifications. The fibre sample weighing 0.5 g was mixed with 25 mL of 50 mmoL/L glucose solution. The mixture was then dialyzed in 80 mL of deionised water at 37°C using a dialysis membrane (Himedia, India) with a cut off molecular weight of 12000 kDa. After 20, 30, 60 and 120 min, the glucose content in the dialysate was determined using a glucose assay kit (Coral Clinical Systems, India). Glucose solution (50mmoL/L) was taken as control and cellulose was taken as a standard for comparison purpose.

The GDRI was calculated from the glucose content in control and fibre samples.

$$GDRI = \left[100 - \frac{G_1}{G_2}\right] \times 100 \qquad \textbf{Eq. 7.13}$$

Where, $G_1$ is the glucose content with fibre and $G_2$ means the glucose content of the control.

### 7.2.8. Statistical Analysis

All experiments were carried out at least in triplicates and reported as mean ± standard deviation of mean (S.E.M) using SPSS version 11.5. The data were statistically analyzed by Duncan's multiple range tests at $p \leq 0.05$significance level.

## 7.3. Results and discussion

### 7.3.1. Proximate composition

The proximate values for the fibre samples are given in Table 7.1. The crude protein in WMPL and CMPM was high with 16.45 g/100g and 13.65 g/100g, respectively. Crude lipid for the fibre samples ranged from 1.43-3.73 g/100g with highest content in KMPL and WMPL samples. The BB fibre sample showed highest ash content. Overall, these fibre samples were found to be good sources of protein and minerals.

**Table 7.1.** Moisture content and proximate composition of the obtained fibre samples

| Sample | Moisture content (g/100g, as is) | Crude protein (g/100g, db) | Crude lipid (g/100g, db) | Ash content (g/100g, db) |
|---|---|---|---|---|
| PNPM | 4.45±0.21$^{b}$ | 5.95±0.16$^{a}$ | 1.43±0.10$^{a}$ | 1.93±0.08$^{a}$ |
| BGPL | 3.59±0.19$^{a}$ | 9.10±0.11$^{b}$ | 1.53±0.15$^{b}$ | 6.86±0.12$^{b}$ |
| KMPL | 6.81±0.11$^{c}$ | 8.05±0.18$^{b}$ | 3.73±0.11$^{e}$ | 2.31±0.09$^{a}$ |
| CMPM | 9.75±0.12$^{e}$ | 13.65±0.10$^{d}$ | 1.94±0.08$^{c}$ | 2.84±0.15$^{a}$ |
| WMPL | 7.65±0.17$^{d}$ | 16.45±0.18$^{e}$ | 3.32±0.20$^{d}$ | 7.86±0.17$^{c}$ |
| BB | 7.83±0.23$^{d}$ | 10.50±0.11$^{c}$ | 1.58±0.11$^{b}$ | 11.67±0.11$^{d}$ |

* Means with the same letter between the rows are not significantly different at $p \leq 0.05$ by DMRT
# PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

### 7.3.2. Yield and total dietary fibre

The fibre samples after defatting with hexane and treating with 80% hot alcohol were calculated for their yield (Table 7.2). Yield was higher for BGPL and BB than the rest. PNPM contained highest total dietary fibre (79.76 g/100g). In all samples, insoluble fibre content (28.57-62.21 g/100g) was more than soluble fibre (9.23-17.55 g/100g), which indicates similarity in composition of the native non starch carbohydrate in these samples.

**Table 7. 2.** Yield and dietary fibre of the obtained fibre samples

| Sample | Yield ( g/100g ) | TDF (g/100g) | IDF ( g/100g ) | SDF ( g/100g ) |
|---|---|---|---|---|
| PNPM | 66.07±0.48$^{c}$ | 79.76±0.42$^{d}$ | 62.21±0.33$^{e}$ | 17.55±0.11$^{c}$ |
| BGPL | 79.94±0.41$^{d}$ | 67.27±0.39$^{e}$ | 56.41±0.38$^{d}$ | 10.86±0.17$^{a}$ |
| KMPL | 52.83±0.14$^{a}$ | 37.82±.33$^{a}$ | 28.57±0.41$^{a}$ | 9.23±0.23$^{a}$ |
| CMPM | 62.08±0.23$^{c}$ | 60.17±0.29$^{d}$ | 46.32±0.22$^{c}$ | 13.84±0.27$^{b}$ |
| WMPL | 58,07±0.19$^{b}$ | 47.48±0.47$^{c}$ | 32.45±0.25$^{b}$ | 15.03±0.14$^{c}$ |
| BB | 77.18±0.20$^{d}$ | 42.12±0.35$^{b}$ | 29.21±0.19$^{a}$ | 12.91±0.25$^{b}$ |

* Means with the same letter between the rows are not significantly different at $p \leq 0.05$ by DMRT
# PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

### 7.3.3. Colour

The hunter colour values varied among the samples (Table 7.3). In the studied fibre samples, the 'L' value ranged from 54.06-77.12. Likewise, five fibre samples showed positive 'a' values and in WMPL where a negative 'a' value was observed indicating that WMPL was slight green in colour that could be due to residual chlorophyll content. Similarly, the 'b' value ranged from 6.77 to 24.58 for the six samples. KMPL showed more inclination towards the yellow colour while BB showed the lowest 'b' value.

### 7.3.4. Physicochemical properties

The physicochemical properties of the fibre samples are mentioned in the Table 7.4. The bulk density of the fibres was quite comparable with that of cellulose taken as control (0.48 ±0.03 g/mL). The water holding capacity (WHC) was high in KMPL, WMPL and BB. It was observed that the oil holding capacity (OHC) was more in BGPL and KMPL. Both WHC and OHC were significantly greater in the studied samples as compared to cellulose (4.47 ± 0.21 g/g and 3.05 ± 0.08 g/g, respectively).

**Table 7.3.** Colour values *(L, a, b)* of the fibre samples

| Sample | *L* | *a* | *b* |
|---|---|---|---|
| PNPM | 67.74±0.21$^{b}$ | 1.84±0.11$^{b}$ | 11.08±0.18$^{b}$ |
| BGPL | 59.50±0.19$^{a}$ | 7.15±0.09$^{d}$ | 14.95±0.32$^{c}$ |
| KMPL | 77.12±0.11$^{c}$ | 4.14±0.12$^{c}$ | 24.58±0.20$^{d}$ |
| CMPM | 65.96±0.13$^{b}$ | 3.55±0.16$^{c}$ | 15.47±0.22$^{c}$ |
| WMPL | 64.45±0.16$^{b}$ | -2.81±0.12$^{a}$ | 14.27±0.17$^{c}$ |
| BB | 54.06±0.19$^{a}$ | 2.01±0.09$^{b}$ | 6.77±0.14$^{a}$ |

* Means with the same letter between the rows are not significantly different at p≤0.05 by DMRT
# PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

Lastly, the swelling capacity of WMPL and BB was high at 16.97 mL/g and 13.97 mL/g, respectively. The swelling capacity of all the samples was comparable with that of cellulose (10.40 mL/g± 0.39). WHC and swelling capacity indicates the hydration properties of the fibre and gives an insight into the behavior of the fibre in a particular food system which is a vital parameter while developing a new product development as well as during

their transit through the gastric tract. Fibre with good hydration property will hold more water and thus contribute to the fecal bulking process. [18] Similarly, good oil holding property indicates reduced absorption of fat in intestine and increased excretion. This may play a positive role in lowering serum cholesterol as well as reduce the deposition of cholesterol in the aorta and liver.

### 7.3.5. Phytochemical content and antioxidant activity

Dietary fibre derived from by-products of fruits and vegetables contains significant amount of phytochemicals in bound form within the fibre matrix and are released inside the human gut on action of the digestive enzymes and colonic bacterial fermentation. [3, 19] In the present study, all the fibre samples showed good phytochemical and antioxidant activity (Table 7.5). Relatively, the highest total phenolic content (TPC) was observed in CMPM followed by BGPL, KMPL and BB. PNPM and WMPL had low TPC values compared to the rest of the samples. Highest total flavonoid content (TFC) was observed in KMPL followed by CMPM and WMPL. Perez-Jimenez and Saura-Calixto [20] reported that polyphenols concentration in grape antioxidant dietary fibre was 19740 mg/100g dry weight. Rubilar et al. [21] found glycosylated flavonols (quercetin, kaempferol) in extracts of white and red grape pomace. Likewise, Schieber et al. [22] mentioned the presence of hesperidin and eriocitrin in peel and solid residue of lemon waste.

**Table 7.4**. Physicochemical properties of the fibre samples

| Sample | Bulk density (g/mL) | Water holding capacity (g/g) | Oil holding capacity (g/g) | Swelling capacity (mL/g) |
|---|---|---|---|---|
| PNPM | $0.29\pm0.09^{a}$ | $9.49\pm0.08^{d}$ | $7.85\pm0.11^{c}$ | $11.93\pm0.11^{c}$ |
| BGPL | $0.30\pm0.06^{a}$ | $7.56\pm0.10^{c}$ | $10.64\pm0.16^{f}$ | $9.40\pm0.13^{a}$ |
| KMPL | $0.46\pm0.05^{c}$ | $13.96\pm0.12^{e}$ | $12.00\pm0.12^{g}$ | $10.30\pm0.07^{b}$ |
| CMPM | $0.46\pm0.03^{c}$ | $6.83\pm0.17^{b}$ | $8.60\pm0.09^{d}$ | $9.39\pm0.20^{a}$ |
| WMPL | $0.39\pm0.10^{b}$ | $15.29\pm0.11^{g}$ | $6.06\pm0.19^{b}$ | $16.97\pm0.25^{e}$ |
| BB | $0.50\pm0.05^{d}$ | $14.17\pm0.19^{f}$ | $9.33\pm0.14^{e}$ | $13.97\pm0.18^{d}$ |
| CEL | $0.48\pm0.03^{e}$ | $4.47\pm0.21^{a}$ | $3.05\pm0.08^{a}$ | $10.40\pm0.39^{b}$ |

* Means with the same letter between the rows are not significantly different at $p\leq0.05$ by DMRT

# PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

The antioxidant activity of the fibre samples were determined by FRAP and DPPH methods. FRAP values in the fibre ranged from 685.76- 12250.00 μmoL/100g. The DPPH radical scavenging activity values ranged from 25.58-94.30%. The metal chelation activity ranged from 1.28-11.68 %. Overall, KMPL, BGPL, CMPM and BB showed good residual phytochemical content as well as antioxidant activity.

**Table 7.5.** Phytochemical content and antioxidant activities of the fibre samples

| Samples | TPC (mgGAE/100g) | TFC (mgQE/100g) | FRAP (μm/100g) | DPPH (%) | MCC (%) |
|---|---|---|---|---|---|
| PNPM | 368.50± 0.45$^{b}$ | 60.38±0.23$^{b}$ | 2868.06±0.27$^{b}$ | 68.56±0.11$^{b}$ | 2.21±0.21$^{b}$ |
| BGPL | 1029.00±0.39$^{f}$ | 69.88±0.11$^{c}$ | 6006.94±0.33$^{d}$ | 91.98±0.17$^{d}$ | 1.28±0.17$^{a}$ |
| KMPL | 867.50±0.21$^{d}$ | 251.75±0.17$^{f}$ | 5833.33±0.41$^{c}$ | 93.72±0.20$^{e}$ | 3.49±0.11$^{c}$ |
| CMPM | 989.00±0.27$^{e}$ | 91.85±0.25$^{e}$ | 12250.00±0.47$^{f}$ | 94.30±0.14$^{e}$ | 8.83±0.14$^{f}$ |
| WMPL | 182.00±0.18$^{a}$ | 73.88±0.15$^{d}$ | 685.76±0.42$^{a}$ | 25.58±0.19$^{a}$ | 5.27±0.09$^{e}$ |
| BB | 433.00±0.14$^{c}$ | 33.75±0.19$^{a}$ | 6791.66±0.39$^{e}$ | 87.50±0.21$^{c}$ | 4.49±0.08$^{d}$ |

* Means with the same letter between the rows are not significantly different at p≤0.05 by DMRT

# PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

### 7.3.5.1. *Phenolic acids compositions of the selected fibres determined by RP-HPLC*

The identified phenolic acids composition varied depending on the fibre source and only the results obtained at 254 nm are given and discussed as the peak intensity of the phenolic acids identified were not very low at 325 nm (Table 7.6 and Fig.7.1 ). Gallic acid (RT=3.23 min), catechin (RT=11.89 min), chlorogenic acid (RT=13.54 min), caffeic acid (RT=14.49 min), syringic acid (RT=14.73 min), ferulic acid (RT=16.55), coumaric acid (RT=16.72 min), rutin (RT=17.31 min) and quercetin (RT=19.89 min) were present in some of the six fibre samples. Gallic acid was predominantly present in all the six samples. The highest content was reported in BB sample (27.00±0.10 mg/100g). Catechin was present in CMPM, BGPL and WMPL. Chlorogenic acid was present only in WMPL. Similarly, KMPL showed highest caffeic acid content (53.09±0.12 mg/100g) followed by PNPM (11.09±0.09 mg/100g). In rest of the samples caffeic acid was not detected. Likewise, syringic acid was highest in KMPL (22.94±0.09 mg/100g) while CMPM and BGPL showed no detection.

Ferulic acid was present in all the samples except in WMPL and KMPL. Similarly, coumaric acid was present only in BGPL and WMPL. The rutin hydrate content was highest in KMPL sample. BB and CMPM samples also showed the presence of rutin hydrate. Lastly, CMPM, BGPL and BB samples exhibited the presence of low amount of quercetin in them. Differences in phenolic acids compositions in fruits are dependent on type of samples, environmental conditions, locations and agronomic factors, maturity stages and type of processing techniques. [23] Therefore, it can be inferred that, even after processing treatments given during the fibre extraction, the samples retained reasonable amount of phenolic acids ranging from low to high consisting of hydroxybenzoic and hydroxycinnamic acid derivatives and flavonoids. Comparatively good retention was observed in KMPL, BB, CMPM followed by PNPM and thus these fibre samples could be referred to as antioxidant dietary fibre.

**Table 7.6.** Phenolic acids (mg/100g) compositions of the selected fibres determined by RP-HPLC

| Sample | GA* | CTH* | CGA* | CFA* | SA* | FA* | CMA* | RH* | QTH* |
|---|---|---|---|---|---|---|---|---|---|
| PNPM | 7.61±0.09 | ND | ND | 11.09±0.09 | 0.63±0.04 | 0.12±0.01 | ND | ND | ND |
| BGPL | 0.46±0.11 | 2.33±0.05 | ND | ND | ND | 1.14±0.03 | 1.28±0.07 | ND | 0.43±0.02 |
| KMPL | 7.07±0.02 | ND | ND | 53.09±0.12 | 22.94±0.09 | ND | ND | 38.62±0.08 | ND |
| CMPM | 6.50±0.09 | 10.72±0.05 | ND | ND | ND | 13.02±0.06 | ND | 1.47 | 1.22±0.05 |
| WMPL | 6.39±0.14 | 2.46±0.02 | 4.53±0.05 | ND | 2.01±0.02 | ND | 4.53±0.05 | ND | ND |
| BB | 27.00±0.10 | ND | ND | ND | 4.68±0.05 | 4.83±0.02 | ND | 7.86±0.03 | 1.43±0.07 |

* Results are mean of triplicates values; GA- gallic acid; CTH- catechin; CGA- chlorogenic acid; CFA- caffeic acid; SA- syringic acid; FA- ferulic acid; CMA- coumaric acid; RH- rutin hydrate; QTH- quercetin

# PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

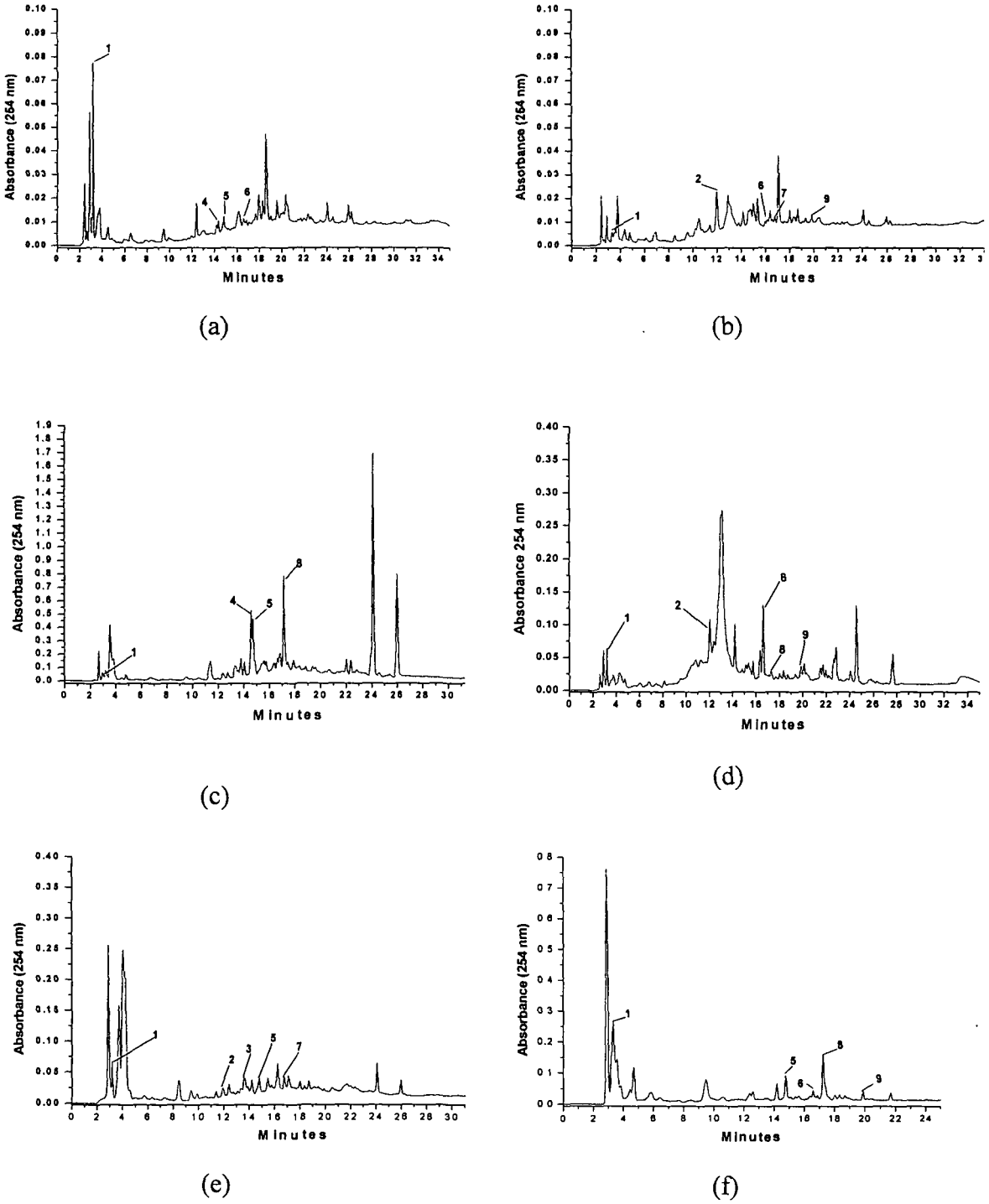


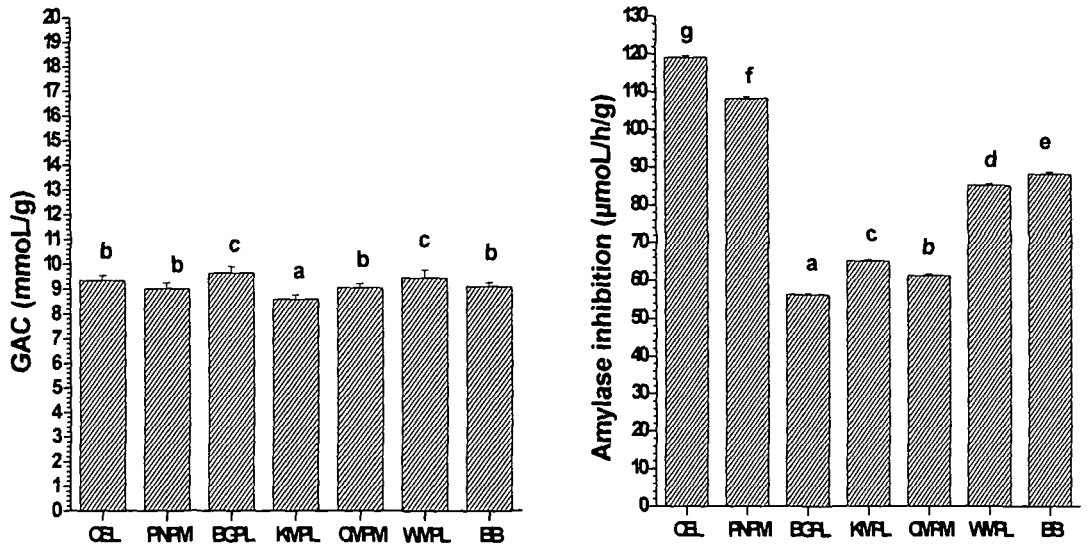

**Fig. 7.1.** Phenolic acid compositions determined by RP-HPLC of (a) PNPM (b) BGPL (c) KMPL (d) CMPM (e) WMPL and (f) BB fibre samples at 254 nm. PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

**Footnote**: 1-gallic acid; 2- catechin; 3- chlorogenic acid; 4- caffeic acid; 5- syringic acid; 6- ferulic acid; 7- coumaric acid; 8- rutin hydrate and 9- quercetin

### 7.3.6. Functional properties

#### 7.3.6.1. *Glucose adsorption and amylase activity inhibition rate*

The adsorption of glucose by the different fibres ranged from 8.57- 9.62 mmoL/g (Fig 7.2 a). All the fibre samples showed adsorption capacity comparable to that of cellulose (9.33 mmoL/g). It can be seen from Fig. 2 (b) that all the fibre samples showed significant variation in amylase inhibition rate with values ranging from 56-108 μmoL/h/g.




(a) (b)

**Fig. 7. 2(a)** Glucose adsorption capacity and (b) amylase inhibition of the fibre sample. PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom. Same letter between the bars means no significant difference at $p \leq 0.05$ by DMRT.

Only PNPM showed inhibition of 108 μmoL/h/g which was comparable with that of cellulose taken as control (119 μmoL/h/g). Lowest inhibition was observed in BGPL. The inhibition of amylase is usually dependent on the insoluble fibre content. However, in the present study, BGPL and CMPM with high insoluble dietary fibre showed low inhibition of amylase whereas in comparison, WMPL and BB with low insoluble dietary fibre exhibited higher amylase inhibition rate. This exception could be due to variation in the molecular weight of the fibre component depending on the source. Other possible reason for amylase inhibition not being in accordance with the insoluble dietary fibre might be attributed to the

reduced accessibility of the enzyme to their substrate due to the encapsulation of starch and enzyme by the fibre. [17, 24]

Amylase inhibition property of the fibre samples indicates their positive role in delaying the rate of glucose absorption in the intestine due to delayed release of glucose from starch digestion which ultimately lowers postprandial serum glucose. [25]

### 7.3.6.2. *Glucose diffusion rate (GDR) and glucose diffusion reduction index (GDRI)*

The glucose diffusion rate indicates the release of glucose during the metabolic processes in the human gut and its subsequent absorption by the body. The fibre in diet plays a crucial role in controlling the rate of glucose diffusion. The glucose diffusion rate thus determines the GDRI. The GDRI measures or predicts the role of fibre in reducing the glucose absorption in the GI tract. [26] Table 7.7 shows the GDR and GDRI values for the studied samples. GDR was slower in BGPL, CMPM and BB compared to cellulose, PNPM, WMPL and KMPL. GDR was inversely proportional to GDRI and also, GDRI values decreased with increase in time. The decrease in GDRI was highest in PNPM, but CMPM showed relatively less decrease in GDRI value after 30 min incubation time.

**Table 7.7.** Glucose diffusion rate and GDRI of the selected fibres

| Sample | 20 min | | 30 min | | 60 min | | 120 min | |
|---|---|---|---|---|---|---|---|---|
| | GDR (μmoL) | GDRI | GDR (μmoL) | GDRI | GDR (μmoL) | GDRI | GDR (μmoL) | GDRI |
| Control | 138.00±0.18$^{d}$ | 0 | 195.00±0.42$^{e}$ | 0 | 280.00±0.45$^{g}$ | 0 | 371.00±0.33$^{g}$ | 0 |
| CEL | 119.00±0.20$^{c}$ | 13.77$^{b}$ | 157.00±0.33$^{b}$ | 19.44$^{d}$ | 258.00±0.52$^{e}$ | 7.86$^{a}$ | 332.00±0.45$^{d}$ | 10.51$^{c}$ |
| PNPM | 125.00±0.24$^{c}$ | 9.42$^{b}$ | 189.00±0.31$^{e}$ | 3.08 $^{a}$ | 260.00±0.13$^{f}$ | 7.14$^{a}$ | 357.00±0.23$^{f}$ | 3.71$^{a}$ |
| BGPL | 100.00±0.09$^{b}$ | 27.54$^{c}$ | 155.00±0.24$^{b}$ | 20.51$^{d}$ | 220.00±0.11$^{b}$ | 21.43$^{d}$ | 306.00±0.27$^{b}$ | 17.52$^{e}$ |
| KMPL | 134.00±0.12$^{d}$ | 2.90$^{a}$ | 184.00±0.27$^{d}$ | 5.64 $^{b}$ | 246.00±0.25$^{d}$ | 12.14$^{b}$ | 359.00±0.49$^{f}$ | 3.23$^{a}$ |
| CMPM | 104.00±0.11$^{b}$ | 24.64$^{c}$ | 163.00±0.11$^{c}$ | 16.41$^{c}$ | 235.00±0.34$^{c}$ | 16.07$^{c}$ | 317.00±0.37$^{c}$ | 14.56$^{d}$ |
| WMPL | 122.00±0.10$^{c}$ | 11.59$^{b}$ | 183.00±0.16$^{d}$ | 6.15 $^{b}$ | 255.00±0.38$^{e}$ | 8.93$^{a}$ | 343.00±0.26$^{e}$ | 7.55$^{b}$ |
| BB | 78.00±0.23$^{a}$ | 43.48$^{d}$ | 132.00±0.23$^{a}$ | 32.31$^{e}$ | 210.00±0.21$^{a}$ | 25.00$^{e}$ | 295.00±0.3$^{1a}$ | 20.48$^{f}$ |

* Means with the same letter between the rows are not significantly different at p≤0.05 by DMRT.

#PNPM- pineapple pomace; BGPL- Burmese grape peel; KMPL- Khasi mandarin peel; CMPM- carambola pomace; WMPL- watermelon and BB- banana blossom.

Reduction in glucose absorption by the intestine could be due to the viscosity caused by the fibre, mainly the soluble fibre fraction, upon water uptake [26]. Also the individual glucose adsorption capacity of the fibre may contribute to the final glucose absorption rate in the GI tract. Another reason could be the entrapment of glucose molecule between the networks formed by fibres [28] which in turn acted as a physical barrier towards the glucose absorption process.

Lastly, good glucose adsorption, amylase inhibition and high GDRI properties in the selected fibres would allow them to be incorporated or fortified as a functional fibre in the development of low calorie and glucose lowering food products.

### 7.4. Conclusion

The present study of the six fibre samples showed varied proximate, physicochemical, phytochemical and functional properties. WMPL and BB were rich in crude protein and ash content. Even though PNPM had highest amount of dietary fibre, other samples showed comparatively better results for all the parameters tested. All the samples showed good water and oil holding capacity as well as swelling capacity. The fibre samples contained good phytochemical content and antioxidant activity. CMPM contained highest TPC and FRAP values. The samples showed considerably good values for glucose adsorption capacity, amylase inhibition, glucose diffusion rate and GDRI. Among the six samples, BGPL, BB, CMPM showed high GDRI values. All the selected fibres can be suitably used as sources of dietary fibre and antioxidants for development of low calorie, high fibre functional food products in the food industries. *In vivo* studies could be carried out to determine the health promoting properties of these fibres.

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