Effect of Gamma Radiation on Morphological, Thermal and Physico-chemical Properties of Dietary Fiber Extracted from Pineapple shell

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Abstract. Present work was conducted to investigate the effects of gamma radiation on morphological, thermal and physico-chemical properties of dietary fiber extracted from pineapple-shell. Water retention capacity of treated (20 kGy) and untreated dietary fiber were 4.3 ± 0.34 and 4.9 ± 0.3 g/g respectively. Dietary fiber treated with 20 kGy showed higher water swelling capacity (12.9± 0.3 mL/g) than un-irradiated fiber (9.13± 0.1 mL/g). Fat and glucose binding capacity were found significantly higher in irradiated sample compared to control. Both differential scanning calorimetry and thermo gravimetric analysis confirmed that the thermal stability of dietary fibers decreased with increased radiation doses.

Keywords: Dietary fiber, Gamma irradiation, Fat binding capacity, Glucose binding capacity.

1 INTRODUCTION

Plant's by-products of food processing causes a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used because of their favorable technological or nutritional properties (Jurasová et al., 2011). The interest in dietary fiber rich food increased in the recent decades, and the demand of this food component had led to the development of a large market for fiber-rich products and ingredients (Jurasová et al., 2011). Dietary fiber is plant-derived material or corresponding carbohydrates that are resistant to digestion in the small intestines, but undergoes complete or incomplete fermentation in the large intestines (Daou and Zhang, 2012). Dietary fiber behaves within the gastrointestinal tract as a polymer matrix with variable physicochemical properties including water-holding capacity, cation-exchange, and adsorptive functions (Căpriţă et al., 2010). A high dietary fiber intake has numerous health benefits including reduced risk of coronary heart disease, diabetes, obesity and some forms of cancer (Mann and Cummings, 2009). Important physicochemical characteristics of fiber is hydration properties which explain the biological effects, namely induction of colonic fermentation and increase in stool weight (Stephen and Cummings, 1979 and McBurney et al., 1985) and it also may affect the texture of food products (Auffret et al., 1994). Different hydration characteristic of dietary fiber are: The swelling capacity which quantify the volume occupied by the hydrated fibers (Kuniak and Marchessault, 1972), the water-binding capacity (WBC) measures the amount of water held after using an external force such as centrifugation (MacConnell et al., 1974) and the water-holding capacity (WHC) measure water held by fibers without stress (Chen et al., 1984).

Different processes such as grinding; drying, heating and extrusion can modify the physical properties of fiber matrix and thus consequently affect the hydration properties (Guillon and Champ, 2000). Conditions of preparation of fiber (temperature and time of soaking), methods used (nature and strength of the external force applied), conditions of measurement (pH, ionic strength), may modify the values of hydration properties (MacConnell et al., 1974). More recently, application of micronization technology (i.e. ball milling, jet-milling, high-pressure micronization) seems to improve the physicochemical properties of insoluble fibers from vegetables and fruits (Chau et al., 2006, 2006, 2007). This micron technology decreases the particle size of dietary fiber, thus considerably improving hydration properties of fiber (Mateos-Aparicio et al., 2010). The source of fiber is also important because various arrays of plant cells can affect fiber properties. Pineapple shell, a high-fiber part of pineapple fruit, could be considered as a potential fiber source (Larrauri et al., 1997). Irradiation of foods is now legally recognized in many countries as a safe and effective method for improving food safety (Kume et al., 2009) and it has no toxic residual effects on treated products (Fapohunda et al., 2012). Ionizing radiation like gamma radiation is being used to improve physico-chemical property of polysaccharide like chitosan, Na-alginate etc (Mollah et al., 2009 and Kume et al., 2002). However, no studies were found on the effect of gamma radiation on physico-chemical properties of dietary fiber extracted from pineapple by-products. Thus the present study was attempted to evaluate the effects of gamma radiation (5, 10, 20, 30 and 40 kGy) on the morphological, thermal and physico-chemical properties (water holding capacity, water retention capacity, water swelling capacity, fat and glucose binding capacity) of dietary fiber extracted from pineapple shell.

2 MATERIAL AND METHODS

2.1 Plant Materials

Pineapple (*Ananascomosus L. Merr.*) shells were collected from a local market, Bangladesh and transported to the laboratory of Food Technology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Savar, Dhaka.

2.2 Dietary Fiber Extraction

Pineapple shells were cut into small pieces washed with hot water and cold water to remove contaminants and dried at 100°C for 5 hr to reduce moisture contents.

Dietary fiber was extracted from dried shells by AOAC method (AOAC.Official Method of Analysis, 1970). Dry pineapple shells (5g) were taken in 500 mL beaker and 200 mL boiling (1.25 %) $H2SO_4$ was added and boiled for 30 min. The volume of this mixture was kept constant by adding distilled water at frequent intervals. A glass rod inserted into the beaker for smooth boiling. Then the mixture was filtered through a muslin cloth. The residue was washed with hot water until it became acid free which was tested by litmus paper.

The materials were then placed into a 500 mL beaker and 1.25% NaOH was added and maintained same procedure as above. After filtration, the material was washed with hot water for alkali free which was checked by litmus paper. The residue was washed with 95% ethanol and acetone and dried at 105°C for 6 hr.

2.3 Irradiation

Extracted dietary fiber was dried in oven for one hour at 105°C and for each radiation treatment 10 ± 1g of dietary fibers were placed into polythene bags (25 cm X 15 cm) and sealed tightly. The sealed polythene bags were labeled by indicating the name of the product

and were irradiated with 5 selected doses (5, 10, 20, 30 and 40 kGy) of gamma radiation. Irradiation was applied to the samples with a 50kCi ^{60}Co gamma source (dose rate 6.4 kGy/ hr) located at Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Savar, Dhaka. Both treated and untreated samples were stored in desiccators at room temperature and measured hydration properties of extracted fibers.

2.4 Morphological Analysis of Dietary Fiber by Scanning Electron Microscopy

The phase morphology of non-irradiated and irradiated dietary fiber was investigated by JSM-6490 scanning electron microscope with a high resolution of 3.0 nm. Its asynchronous five-axis mechanically eucentric stage with compeucentric rotation and tilt can accommodate a specimen of up to 8-inches in diameter (12-inches loadable). Standard automated features include Auto Focus/Auto Stigmator, Auto Gun (saturation, bias and alignment), and Automatic Contrast and Brightness.

2.5 Physico-chemical Properties of Extracted Dietary Fiber

Physico-chemical properties including hydration properties (water holding water, retention and swelling capacities), glucose and fat binding capacities of dietary fiber were determined.

2.5.1 Hydration Properties of Extracted Dietary Fiber

Water holding capacity, water retention capacity and water swelling capacity were determined according to Raghavendra *et al.* (2008) (Raghavarao et al., 2008).

2.5.1.1 Water Holding Capacity (WHC)

Control and irradiated fibers (1g) were taken in a test tube and added 30 mL of water and hydrated for 18 h at 37°C. The supernatant was removed by passing through sintered glass crucible (G4) under vacuum, and then the hydrated residues were collected. The residues were weighted and dried at 105°C for 2 hr to obtain residual dry weights.

Water holding capacity (g/g) = (Residue hydrated weight - Residue dry weight) / Residue dry weight

2.5.1.2 Water Retention Capacity (WRC)

Both control and irradiated samples (0.5 g) were taken in a centrifuged tube where 15 mL of water was added and hydrated for 18 h at 37°C, centrifuged (5000 rpm ; 20 min) and the supernatant was discarded by passing through sintered glass crucible (G4) under vacuum. The hydrated residues were weighted and dried at 105°C for 2 hr to obtain residual dry weights.

Water retention capacity (g/g) = (Residue hydrated weight after centrifugation - Residue dry weight) / Residue dry weight

2.5.1.3 Water Swelling Capacity (WSC)

Control and irradiated fibers (0.2 g) were taken in a test tube and 10 mL of water was added. The mixture was hydrated for 18 hr and the final volume attained by fibers was measured.

Swelling capacity (mL/g) = Volume occupied by sample /Original sample dry weight

2.5.2 Fat Binding Capacity (FBC)

The fat binding capacity of control and irradiated fibers were determined by using a modified method of Wang and Kinsella (Wang and Kinsella, 1976). FBC was initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10mL of oil (soybean oil), and mixing on a vortex mixer for 1min to disperse the sample. The contents were left at ambient temperature for 10 min and centrifuged (Model #Z383K, HERMLEJ-National Labnet Company, Woodbridge, NJ.USA) at 3500 rpm (6000x g) for 25 min. After the supernatant was decanted, the tube was weighed again. FBC was calculated as follows:

FBC (%) = Fat bound (g) / Initial sample weight (g) X 100

2.5.3 Glucose Binding Capacity (GBC)

The glucose binding capacity of non-irradiated and irradiated fibers was estimated by using the glucose oxidase method (Barham and Trinder, 1972). Sample (0.4g) was kept in a centrifuge tube, added 10 mL glucose solution and was mixed on a vortex mixer for 1 min for dispersion and then 1.5 mL glucose monoreagent solution was mixed. The contents were left at ambient temperature for 60 min. Blank (only contained glucose monoreagent) and standard (15 µL standard glucose and 1.5 mL glucose monoreagent solution) were run along with samples through the entire procedure. Absorbance was taken at 500 nm by UV-Visible Spectroscopy. GBC was calculated as follows:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = mg/dL \text{ glucose}$$

2.6 Thermal Analysis of Dietary Fiber

Thermal analysis includes Differential scanning calorimetry and Thermo gravimetric analysis.

2.6.1 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were done to understand thermal behavior of dietary fiber. Dietary fiber (3-6 g) was taken in an aluminium pan and pellet was formed in sample preparation machine under pressure. The pellet was then placed in Differential Scanning Calorimeter (Model: DSC-60 Supplier: Shimadzu Corp.). The temperature range was maintained at 30^{o}C to 500^{o}C and the temperature was increased at a rate of 10^{o}C/min. The flow rate of nitrogen gas was 20 mL/min.

2.6.2 Thermo gravimetric analysis (TGA)

A Shimadzu Corp. Model TGA-50 thermo gravimetric system with a microprocessor driven temperature control unit and Thermal Analysis data station, was used to determine the TGA analysis of dietary fiber. The mass of the samples was generally within the range of 1 mg. The aluminium sample pan was placed in the balance system equipment and the temperature was raised from 30 to 600^{o}C at a heating rate of 10^{o}C per minute. The mass of the sample pan was continuously recorded as a function of temperature. The flow rate of nitrogen gas was 10 mL/min.

Effects of radiation on DSC and TGA were observed only for 20 and 40 kGy irradiation doses because significant alteration were found in morphology and physico-chemical properties of the fiber when treated with those two doses.

2.7 Statistical Analysis

Statistical procedures were performed using SPSS for Microsoft version 17.0 software package (SPSS Chicago, IL). To determine significant difference between treatments least significant difference (LSD) at $P = 0.05$ was used.

3 RESULTS AND DISCUSSION

3.1 Effect of Radiation on Morphology

Fig. 1 showed the micrograph of non-irradiated and irradiated (20 and 40 kGy) dietary fibers. The figure revealed that particles of non-irradiated fiber had uniform network and were agglomerated. Closer view (SEM in 1 µm) did not reveal the amorphous structure of the dietary fiber. At 20 kGy, dietary fiber chain was ruptured and irregular arrangements of the molecules were observed. After 40 kGy irradiation, the dietary fiber molecules were degraded more severely and the surface structure of the dietary fiber particles were observed as blistered and scattered. Among all micrographs, significant degradation was found in morphology at 20 and 40 kGy radiation doses.

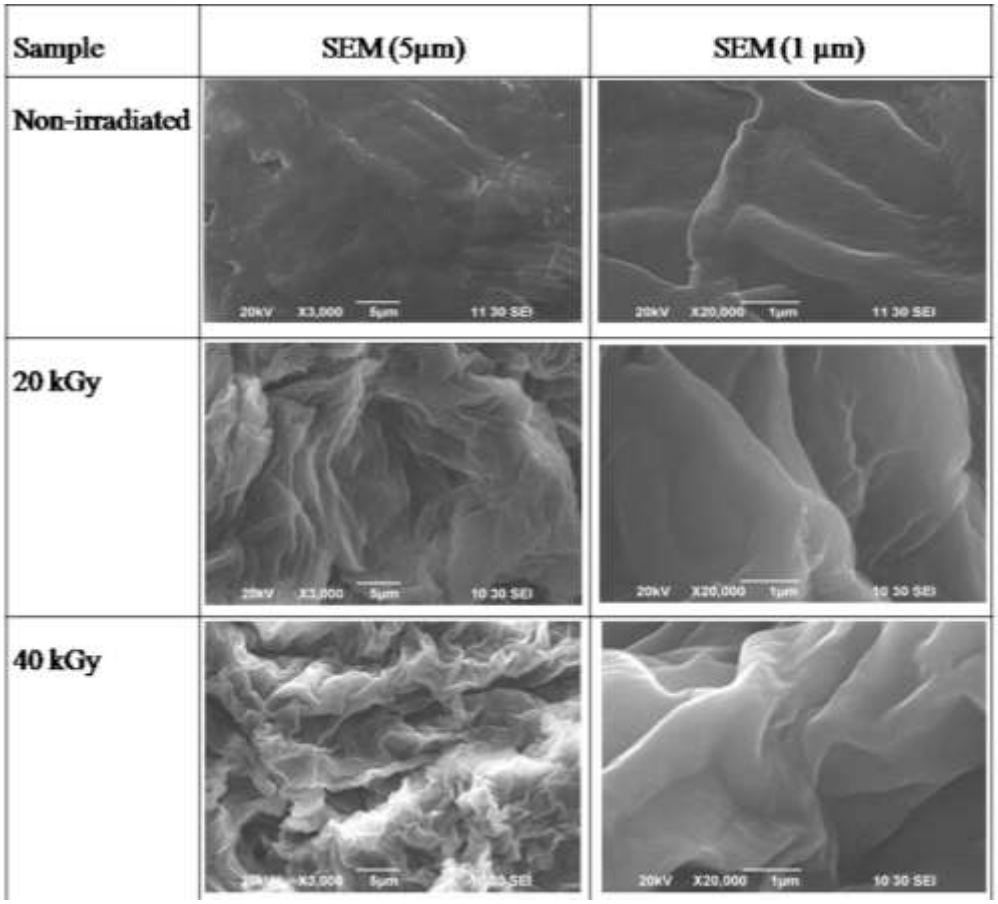

Fig. 1. Scanning electron micrograph of dietary fiber

3.2 Physico-chemical Properties

Hydration properties of dietary fibers may be portrayed by measuring water absorption, water holding and swelling property of fibers which are strongly related to the source of the dietary fiber (Elleuch et al., 2011). Present studies observed the effects of radiation on hydration properties (WHC, WRC and WSC) of dietary fiber extracted from pineapple shell.

Fig. 2 illustrates the WHC, WRC and WSC with respect to gamma radiation doses. It was found that WHC was slightly higher in every radiation treated fiber than control ones (3.69 g/g). Statistical analysis showed that radiation has no significant effect on WHC. Similar results were found in the experiment of Ashraf et al. (2012) who claimed that the microwave treatment (MWT) during all the intervals of time (50, 90 and 300 sec) showed that WHC of red bean flour increased slightly than control sample and MWT at 300 sec showed highest WHC, which may be due to uncoiling and more exposure of the hydrophilic domains of the various proteins, responsible for increase in water absorption.

Regarding the water retention capacity, irradiated fiber did not differ significantly (P = 0.05) from control fiber (4.26 g/g). Likewise, it was found by Mateos et al., (2010) that no marked difference on WRC of fiber of dry okara, by-product from soybean when subjected to high hydrostatic pressure (HHP) and controlled temperature. On the contrary, it was found by Guillon et al. (1992) that WRC of autoclaved sugar-beet fiber was increased by 1.3-fold than control sample.

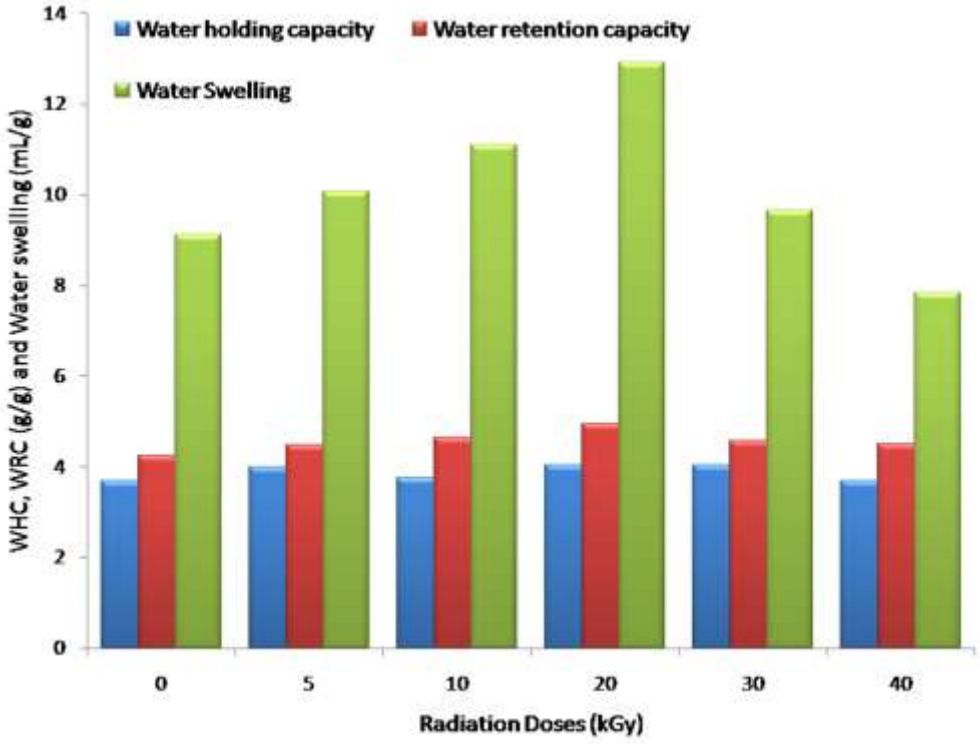

Fig. 2. Effects of radiation on hydration properties of dietary fiber extracted from Pineapple shell (LSD $_{(5\%)}$ was 0.58, 1.12 and 1.42 respectively for WHC, WRC and water swelling)

Statistical analysis of the present results showed that 20 kGy was the most effective radiation dose to increase Water Swelling Capacity (12.92 mL/g) of dietary fiber with 10 kGy also having a greater WSC (11.11 mL/g) than untreated fiber (9.13 mL/g). The increased water swelling capacity of irradiated fiber was probably due to its looser structure, lower density, and the higher number of water-binding sites (Lo et al., 1991). Radiation doses of 5, 30 and 40 kGy have no significant effects on WSC.

Water holding capacity and water swelling capacity suggest some possibilities about the use of fibers as ingredients in food products and high WHC can be used not only as dietary fiber enrichment, but also as functional ingredients to avoid synaeresis and modify the viscosity and texture of some formulated food products (Grigelmo-Miguel and Martin-Belloso, 1999).

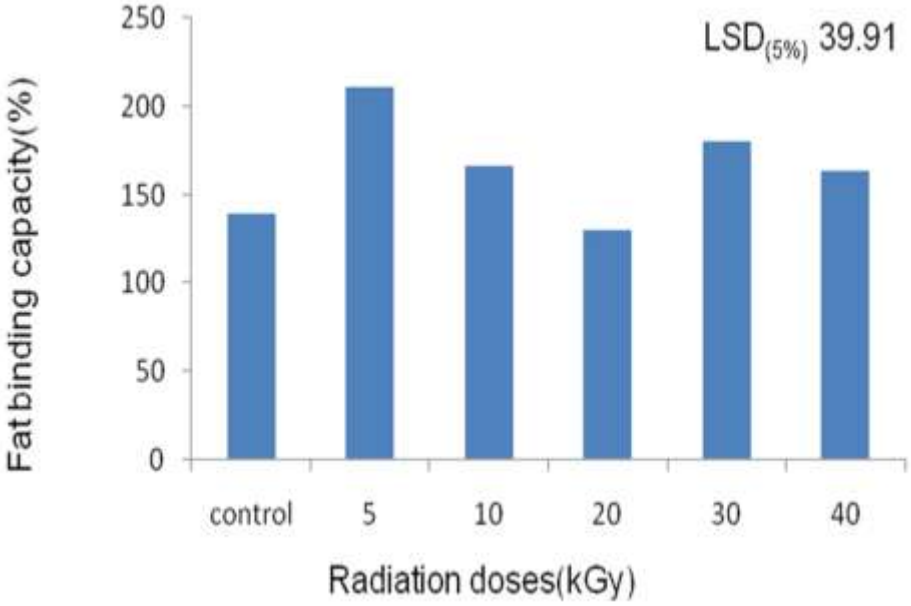

Fig 3. Effects of radiation on fat binding capacity (FBC) of dietary fiber

Effects of radiation on fat binding capacity (FBC) of the dietary fibers were presented in **Fig. 3**. A significantly higher rate of fat binding capacity (FBC) was found in irradiated samples (5, 10, 30 and 40 kGy) compared to control sample. FBC of irradiated fiber is greater than that of un-irradiated because the oil absorption is related to the nature of the surface and the density or thickness of particles, so those particles with the greatest surface area theoretically present a greater capacity to absorb and bind components of an oil nature (Amado, 1994). Difference in fat binding capacity between control and 20 kGy was not significant.

The glucose content of the remaining solution represented the opposite value of glucose binding capacity (GBC). ANOVA statistical analysis showed that significantly higher rate of glucose binding capacity (GBC) was found in all irradiated samples (5, 10, 20, 30 and 40 kGy) compared to control sample **(Fig. 4)**. Glucose binding capacity (GBC) of the dietary fiber was significantly increasing up to 20 kGy and then decreased gradually up to 40kGy. 20 kGy irradiated dietary fiber showed the highest glucose binding capacity among the all treatments may be due to its high viscosity which might improve glucose entrapment within the fiber matrix (Lopez et al., 1996).

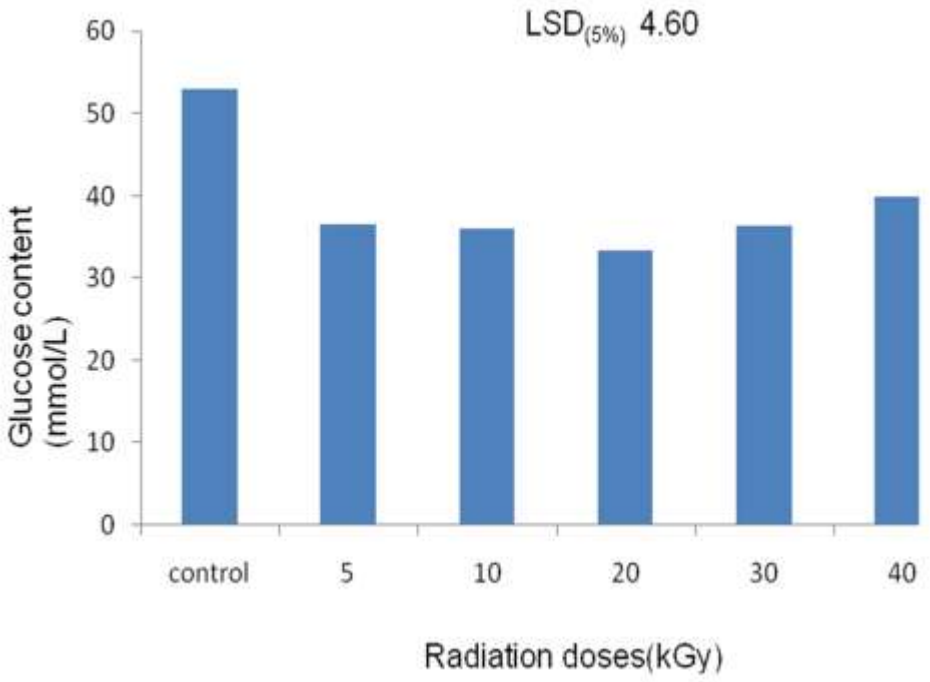

Fig. 4. Effects of radiation on glucose binding capacity (GBC) of dietary. The glucose content of the remaining solution represented the opposite value of glucose binding capacity (GBC)

3.3 Thermal Analysis

Differential scanning calorimetry (DSC) studies were performed to understand the behavior of the dietary fiber on application of thermal energy. Polysaccharides usually have a strong affinity for water and in solid state these macromolecules may have disordered structures that can be easily hydrated. The hydration properties of polysaccharides depend on primary and supra molecular structures. **Fig. 5A** shows the DSC thermogram of dietary fiber within the temperature range of 30 °C to 600 °C at a heating rate of 10 °C/min. Two different types of sharp thermal transition were observed in the thermogram. The wide endothermic peak at temperatures 39.17°C was a result of the loss of moisture within the samples, while the wide exothermic peak at the high temperature region 482.38°C was the result of the thermal decomposition of the samples. By careful consideration of the thermogram of dietary fiber, another transition was observed at 215°C. That endothermic peak probably indicated the glass transition point of the dietary fiber. In fact the glass transition temperature (Tg) of dietary fiber is still a subject of controversy. The main reason may be that, being a natural polymer, some properties like crystallinity, molecular weight and deacetylation degree, can present wide variations according to the source and /or method of extraction and will influence the Tg.

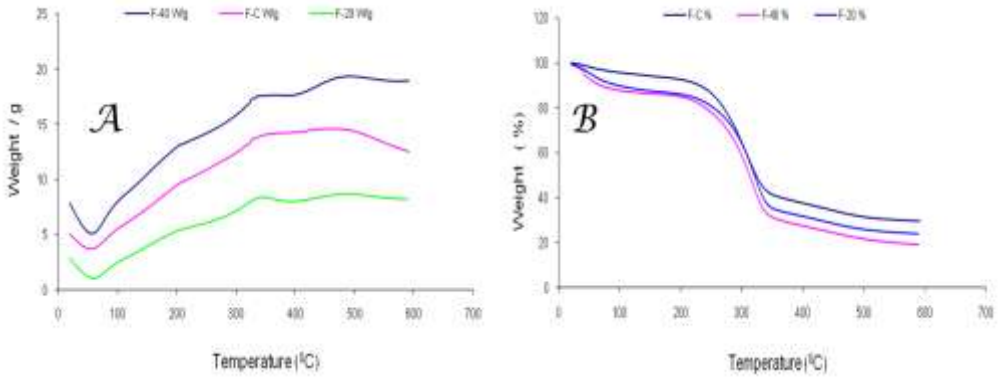

Fig. 5. Effect of radiation (20 and 40 kGy) on thermal analysis, (A. DSC analysis and B. TGA analysis)

A comprehensive study of TGA of the dietary was carried out by thermo gravimetric analyzer (supplier: Shimadzu, Japan, model: TGA-50) within the temperature range 30° to 600°C which revealed its thermal stability and thermal behavior. On **Fig. 5B**, the weight loss at 30°-150°C was due to the moisture vaporization. The other weight loss at 20°-500°C was due to the degradation of dietary fiber. In degradation zone, the point at temperature 215°C indicated the glass transition point of the dietary fiber. The weight loss at 500°C was up to 80%. The reasons of such stability of dietary fiber were intra and inter molecular hydrogen bond between dietary fiber molecules. Gamma radiation dose performed some structural change of dietary fiber. Differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA) were studied to evaluate the thermal stability and miscibility of dietary fiber. The comparison among the thermograms of irradiated dietary fiber revealed that the exothermic peaks (decomposition temperature) within the range 470°-490°C slightly moved to the left with increasing radiation dose i.e. the decomposition temperature of dietary fiber decreased with the increasing radiation dose. The same result was observed in case of glass transition points (endothermic peak in the middle) within the region 200°-230°C and in the case of moisture removal (broad endothermic peak) within the region 30 °C -70 °C. The changes were more visible in case of glass transition temperature and decomposition temperature rather than in case of moisture loss temperature. The degradation of dietary fiber molecules with gamma radiation was responsible for that. The dietary fiber with lower molecular weight degraded at lower temperature than higher molecular weight dietary fiber. Besides as the dietary fiber molecule decomposed with increasing radiation dose, the degree of crystallinity of dietary fiber was also decreased; which helped to decrease the glass transition temperature of the dietary fiber. Radiation had negligible effect on moisture loss temperature as it did not depend on molecular weight largely. The thermo gravimetric analysis of control and irradiated (20 and 40 kGy) dietary fiber also confirmed the change in degradation temperature. The degradation of irradiated dietary started at least 50°C earlier than that of the normal dietary fiber although it was slower in case of irradiated dietary fiber. There was slight transition in degradation temperature from 20 to 40 kGy. The DSC thermograms also revealed the same results. So DSC and TGA confirmed that the thermal stability of dietary fibers decreased with increased radiation dose.

4 CONCLUSION

To achieve optimum benefits to improve physico-chemical, thermal and morphological properties of dietary fiber by gamma radiation, different doses were applied on dietary fiber

extracted from pineapple shell. Irradiation treatments significantly increased water swelling capacity, fat binding and glucose binding capacity that can be helpful to reduce fat and glucose and thus can avoid increase in postprandial blood glucose level as well as lipid content. Therefore, irradiated dietary fiber might be incorporated in daily foods as low calorie bulk ingredient and thus can be employed to combat with the most severe healthcare issues of modern world like heart disease and diabetic mellitus.

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