EXTENSION OF SHELF-LIFE OF GREEN PLANTAINS (*Musa paradisiaca L.*) BY GAMMA-IRRADIATION WITH SPECIAL EMPHASIS ON RADIATION INDUCED ALTERATIONS IN CONTENTS OF L-TRYPTOPHAN, SEROTONIN AND MELATONIN

THESIS SUBMITTED FOR PARTIAL FULFILLMENT OF THE REQUIREMENT

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By

DIPSHIKHA TAMILI

Under the Guidance of

Dr. PARAMITA BHATTACHARJEE

Associate Professor

FACULTY OF ENGINEERING AND TECHNOLOGY

Department of Food Technology and Biochemical Engineering

Jadavpur University

Kolkata-700032

THIS THESIS WORK IS DEDICATED TO MY FAMILY.

Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as a part of her M.Tech. (Food Technology and Biochemical Engineering) studies.

All the information in this document has been obtained and presented in accordance with academic rules and ethical contact. I also declare that, as required by these rules and conduct.

I have fully cited and referenced all materials and results that are not original to this work.

Name: DIPSHIKHA TAMILI

Roll Number: M4FTB19005

Thesis Title: EXTENSION OF SHELF-LIFE OF GREEN PLANTAINS (*Musa paradisiaca L.)* BY GAMMA-IRRADIATION WITH SPECIAL EMPHASIS ON RADIATION INDUCED ALTERATIONS IN CONTENTS OF L-TRYPTOPHAN, SEROTONIN AND MELATONIN

Signature with date:

DEPARTMENT OF FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING

FACULTY OF ENGINEERING AND TECHNOLOGY

JADAVPUR UNIVERSITY

KOLKATA-700 032

Certificate of Recommendation

The thesis entitled 'Extension of shelf-life of green plantains (*Musa paradisiaca L.*) by gamma-irradiation with special emphasis on radiation induced alterations in contents of L-tryptophan, serotonin and melatonin' prepared under the supervisor of Dr. Paramita Bhattacharjee by Dipshikha Tamili (Class Roll No. 001710902004, Registration No. 140862 of 2017-2018), a student of M.Tech final year, has been evaluated by us and found satisfactory. It is therefore being accepted for the partial fulfillment of the requirement for awarding the degree of Master of Technology in Food Technology and Biochemical Engineering.

Dr. Paramita Bhattacharjee
Associate Professor
Department of Food Technology and Biochemical Engineering
Jadavpur University
Kolkata 700 032

Dr. Dipankar Halder
Head
Department of Food Technology and
Biochemical Engineering
Jadavpur University
Kolkata 700 032

Prof. Chriranjib Bhattacharjee
Dean of Faculty of Engineering and
Technology
Jadavpur University
Kolkata 700 032

DEPARTMENT OF FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING

FACULTY OF ENGINEERING AND TECHNOLOGY

JADAVPUR UNIVERSITY

KOLKATA-700 032

Certificate of Approval

This is to certify that **Ms. Dipshikha Tamili** has carried out research work entitled **'Extension of shelf-life of green plantains (*Musa paradisiaca L.*) by gamma-irradiation with special emphasis on radiation induced alterations in contents of L-tryptophan, serotonin and melatonin'** under my direct supervision in the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata. I am satisfied that she has carried out this work independently and with care and confidence. I hereby recommended that this dissertation be accepted in partial fulfillment of the requirements for the degree of Mater of Technology in Food Technology and Biochemical Engineering.

Dr. Paramita Bhattacharjee
Associate Professor
Dept. of Food Technology and Biochemical Engineering
Jadavpur University,
Kolkata 700 032

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SYNOPSIS

Thesis entitled **"Extension of shelf-life of green plantains (*Musa paradisiaca L.*) by gamma-irradiation with special emphasis on radiation induced alterations in contents of L-tryptophan, serotonin and melatonin"** investigates the effects of gamma(γ)-irradiation on green plantains on the contents of L-tryptophan, serotonin and melatonin in γ-irradiated green plantains and also explores whether shelf-life of the green plantains can be extended by γ-irradiation. Considering phytoremediation properties of plantains, it is hypothesized that these antioxidants would be overproduced in aged plantains to scavenge reactive oxygen species generated by γ-irradiation.

To investigate the former, extraction of the natural antioxidants namely serotonin and melatonin and their precursor L-tryptophan from γ-irradiated plantains has been conducted. The method of quantification of the biomolecules was also validated in this thesis. The shelf life study has been conducted based on analyses of sensory attributes, proximate composition, physicochemical and phytochemical properties of the plantains during storage at ambient conditions.

The thesis has been divided into two chapters. **Chapter 1** provides a general introduction to the dissertation topic and discusses about γ- irradiation of green plantains at different doses and the effect of γ-irradiation in enhancing the contents of biomolecules in green plantains namely L-tryptophan, serotonin and melatonin. It also discusses about the quantification of the aforesaid biomolecules by high performance liquid chromatography (HPLC). To validate the simultaneous quantification of the aforementioned biomolecules, mass transfer modeling was conducted. **Part I** of this Chapter discusses in detail the optimization of γ-irradiation doses for alterations in contents of L-tryptophan, serotonin and melatonin in γ-irradiated green plantains. **Section 1.1.1** is the **Introduction and Review of Literature** section which discusses the importance of L-tryptophan, serotonin and melatonin in both plants and humans and the effects of γ-irradiation on alteration of contents of biomolecules in post-harvest produces, such as fruits, vegetables, nuts and flowers. However, no reports on green plantains on these aspects have been found in literature. The objectives and the need of this research work have therefore been elaborated in this section. **Section 1.1.2, i.e., Materials and Methods** provides details of materials and methodologies adopted to investigate the said objective. This section emphasizes on the

optimization of γ-irradiation conditions for enhancing L-tryptophan, serotonin and melatonin contents in green plantains. Post-irradiation extraction of the above mentioned biomolecules from green plantains were conducted by solid phase extraction method. Quantifications of the plantain extracts were conducted by HPLC. These studies have aided in investigating the changes in contents of aforementioned biomolecules in γ-irradiated green plantains, against a 'control' set. **Section 1.1.3** presents **Result and Discussion** which clearly provides all results obtained in the above study with tables and figures. **Section 1.1.4** presents a **Conclusion** on the effects on γ-irradiation in enhancing abovementioned biomolecules in plantains. **Part II** of Chapter 1 discusses the necessity to validate the HPLC method by mass transfer modeling of L-tryptophan, serotonin and melatonin. **Section 1.2.1** is the **Introduction and Review of Literature** section which discusses about the limitations in simultaneous quantification of the said biomolecules at low levels (µg) and the necessity to assess the mass transfer of the biomolecules in HPLC packed bed in terms of dimensionless numbers namely Reynolds (Re) number, Schmidt (Sc) number and Peclet (Pe) number to validate the HPLC method used. The need of limit of detection (LOD) and limit of quantification (LOQ) of these biomolecules have also been discussed. The objective and need of this part of the study is also mentioned. **Section 1.2.2** is **Materials and Methods** section which provides the details for determination of LOD and LOQ of the aforesaid biomolecules and for evaluation of the mass transfer of the biomolecules in HPLC packed bed in terms of Re, Sc and Pe. **Section 1.2.3** presents **Result and Discussion** which clearly provides all results obtained in the above study. **Section 1.2.4** gives a **Conclusion** on validation of HPLC method, for simultaneous quantification of L-tryptophan, serotonin and melatonin.

Chapter 2 discusses about the enhancement of shelf-life of γ-irradiated green plantains in terms of sensory attributes, proximate analysis, physicochemical and phytochemical properties of plantains during storage. **Section 2.1.1** is the **Introduction and Review of Literature** section which discusses on the impact of γ-irradiation technology in extension of shelf-life of fruits and vegetables. The objective and need of this part of the study is also mentioned. **Section 2.1.2** elaborates the **Materials and Methods** employed for this objective of the investigation. This section emphasizes on the optimization of γ-irradiation doses to enhance the shelf-life of γ-irradiated green plantains. Post gamma-irradiation, sensory attributes, proximate analysis, physicochemical and phytochemical properties of plantains were determined during storage.

These studies have aided in investigating the changes in aforementioned properties in gamma-irradiated green plantains, against a 'control' set. **Section 2.1.3** presents **Result and Discussion** which clearly provides all results obtained in the above study with tables and figures and **Section 2.1.4** provides a comprehensive **Conclusion** of the findings on shelf-life extension of gamma-irradiated plantains.

Chapter 3 presents the **Summary and Future Prospects** of the entire research. It provides an overall inference of all the objectives of this project.

1. INTRODUCTION

The local or *"desi"* variety of Indian plantains (*Musa paradisiaca L.)* of the family *'Musaceae'* is extensively cultivated in West Bengal. Presently, plantains rank as the fourth most important food crop in the world after rice, wheat and maize; and are used in preparation of food, beverages, fermentable sugars, medicines, flavorings and cooked foods (Nelson et al., 2006; Phillip et al., 2009). This agro-commodity is widely popular as the 'cooking banana' and being rich in phytochemicals and also a major source of carbohydrates, has been exploited since ages for its numerous health benefits and as a source of energy. Literature reports including those by Bhattacharjee et al. (2016) and Bravo et al. (2015) states that therapeutic efficacy of plantains is due to presence of the phytohormone serotonin. Since serotonin is reportedly present, it is opined that L-tryptophan and melatonin will also be present in plantains.

These compounds are bioactive and serve numerous benefits to plants, including human. Serotonin besides being a plant neurotransmitter also has significant roles in the regulation of physiological functions such as body temperature, sleep, appetite, behavior and cognitive functions in human (D'souza et al., 2006, Lucki 1998). L-tryptophan, an essential amino acid, through a cascade of biological reactions synthesizes melatonin, via serotonin (Szafranska et al., 2016). Melatonin too is a significant plant biomolecule which also plays key roles in development of human brain, body, regulation of circadian and seasonal rhythms, in the sleep-wake process, in reproduction and in retinal function (Bhattacharjee et al., 2016).

It is reportedly known that the content of bioactive compounds in fruits and vegetables may be *altered* by postharvest treatments such as freezes drying (Abonyi et al., 2002) and by low dose gamma (γ) irradiation (Oufedjikh et al., 2000). It is envisaged that γ-irradiation can possibly stimulate production of reactive oxygen species (ROS) in plantains and thereby would enhance synthesis of these phytoremediator molecules, namely serotonin and melatonin which are potential antioxidants, through free radical mechanisms. This environment-friendly, non-thermal green technology has been less explored for enhancement of bioactive compounds in fruits and vegetables. The current study would investigate enhancement of production of these bioactive phytoremediator therapeutic molecules using low dose irradiation. Previous research conducted by our research team has successfully established that γ-irradiation enhanced production of lutein

by stimulating carotenogenesis in irradiated marigold flowers nearing end of senescence (Pal and Bhattacharjee, 2016*)*.

1.1 Review of literature

1.1.1 Therapeutic potency of green plantains

Musa paradisiaca is a herbaceous plant (up to 9 m long) with a robust tree like pseudostem, a crown of large elongated oval deep-green leaves (up to 365 cm in length and 61 cm in width), with a prominent midrib. Each plant produces a single inflorescence like drooping spike and large bracts opening in succession, ovate, 15-20 cm long, concave, dark red in color and somewhat fleshy. Fruits are oblong, fleshy, 5-7cm long in wild form and longer in the cultivated varieties (Zafar and Akter, 2011).

The fruit of *M. paradisiaca* is traditionally used in diarrhea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout, hypertension and cardiac disease (Ghani, 2003; Khare, 2007). It is also rich in antioxidants such as. caffeic acid hexoside, ferulic acid hexoside, sinapic acid, hydroxycinnamic acid derivative, quercetin, myricetin, rutin, kaempferol, isohamnetin-3-0 rutinoside, epicatechin (Tsamo et al., 2015). Moreover, beneficial bioactive molecules present in plantains, namely the above-mentioned phytoremediator molecules including L-tryptophan confer therapeutic properties to plantains.

1.1.1.1 Beneficial bioactive molecules in plantain

1.1.1.1.1 L-tryptophan

L-tryptophan, an aromatic indolylpropionic acid, was identified in 1901 by Hopkins and Cole. It is one of the eight essential amino acids. Since humans cannot synthesize tryptophan, it must be supplemented through the diet (Richard et al., 2009). It plays an important role in protein synthesis, and is also the precursor of a variety of biologically active compounds including serotonin, melatonin, tryptamine, quinolinic acid and kynurenic acid. In addition, tryptophan is the precursor to the coenzymes NAD and NADP, and can replace niacin as an essential nutrient (Sainio et al., 1995).

Chemical structure of L-tryptophan is shown in Figure 1.

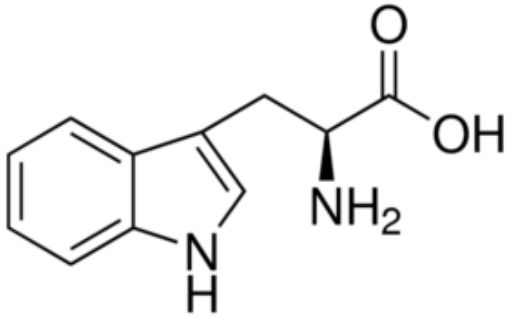

Figure 1: Chemical structure of L-tryptophan

1.1.1.1.2 Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) was discovered 60 years ago in blood, peripheral tissues and in the central nervous system (Erspamer and Asero, 1952) of human. It is a physiologicaliy active amine and a well-known neurotransmitter that regulates mood, sleep and anxiety (Veenstra-VanderWeele et al., 2000). It was first identified as a vasoconstrictor substance that is released from platelets during coagulation of blood, and later as a monoamine neurotransmitter in the brain (Seller and Pivak, 2011). In humans, approximately 90% of serotonin is located in the enterochromaffin cells in the GI tract, where it regulates intestinal movements (Berger et al., 2009). Even it regulates numerous biological processes including cardiovascular functions, bowel motility and bladder control. Chemically serotonin is represented by the formula shown in in Figure 2

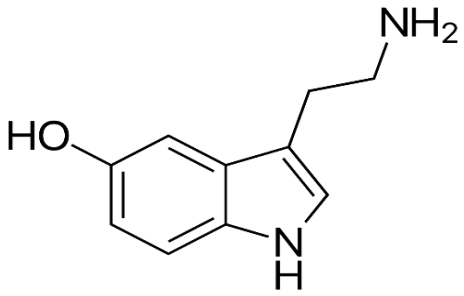

Figure 2: Chemical structure of serotonin

1.1.1.1.3 Melatonin

Melatonin (5-methoxy-N-acetyltryptamine), the 'hormone of darkness' is an indole hormone produced in the pineal gland of brain and controls several functions in our daily lives (Bhattacharjee et al., 2016). Melatonin is an powerful antioxidant (Sahana et al., 2005; Nitulescu-Arsene et al., 2009; Carrillo-Vico et al., 2013; Fatma et al., 2013; Bhavini et al., 2009) and preserves mitochondrial homeostasis, increases gene expression for antioxidant enzymes and thereby is extremely beneficial in redressing neurodegenerative disorders such as Alzheimer's and Parkinson's diseases whose pathogenesis are associated with cytotoxic effects of ROS (Russel et al., 2010; Ayushi et al., 2007; Hardeland, 2005; Jian-zhi ; Ze-fen, 2006, Venkatramanujam, 2011). It has many physiological roles in animals, influencing circadian rhythms, mood, sleep, body temperature, locomotor activity, food intake, retina physiology, sexual behavior (Maronde and Stehle, 2007; Pandi-Perumal et al., 2008; Tan et al., 2012; Hardeland et al., 2012) seasonal reproduction, and the immune system (Carrillo-vico et al., 2013). It also alters the activity of pancreatic cells which is essential for preventing diabetes (Karthikeyan et al., 2014). Chemical structure of melatonin is shown in Figure 3.

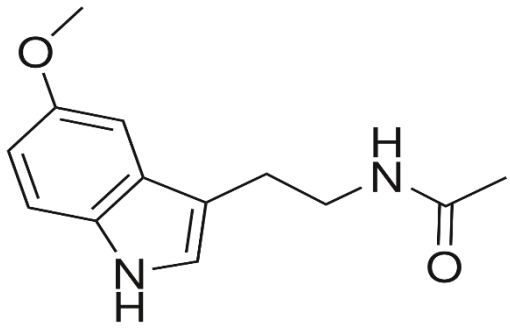

Figure3: Chemical structure of melatonin

The biosynthesis pathway of conversion of serotonin to and melatonin in plants, where L-tryptophan acts as precursor is presented in Figure 4.

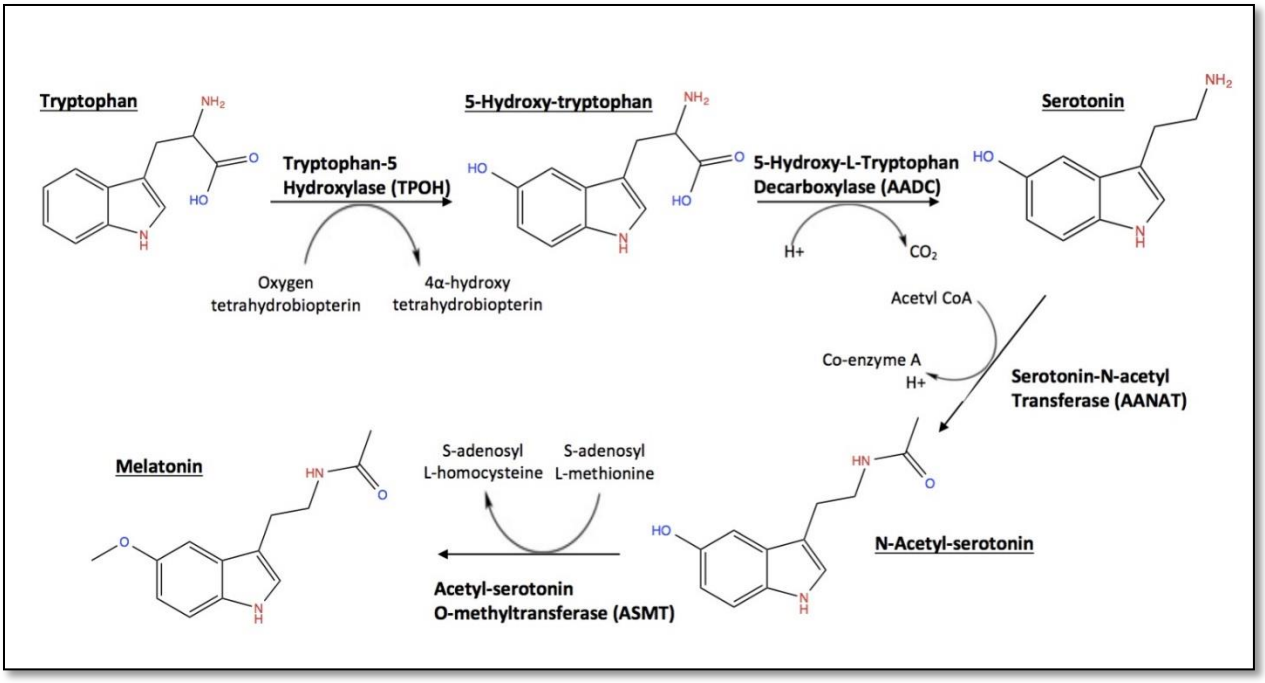

Figure 4: Biosynthesis pathway of production of serotonin and melatonin from their precursor L-tryptophan in plants

A brief literature survey on use of γ-irradiation in post-harvest produces has been provided below.

1.1.2 Effect of γ-irradiation in post-harvest produces

It is observed that depending on the dose of γ-irradiation, biomolecules in post-harvest produce has been altered, even enhanced.

1.1.2.1 Enhanced flavanone and terpenoid contents in 'Rio Red' grape fruit

'Rio Red' grape fruit were subjected to γ-irradiation at doses 0, 0.07, 0.2, 0.4, 0.6 kGy with a centerline absorbed dose of about 0.04 kGy min^{-1}. Post irradiation, the fruits were subjected to simulated storage conditions for 4 weeks at 10°C followed by one week at 20°C, RH 90-95%. Fruits from each treatment were collected for extraction and analysis of phytochemical levels 24 h after γ-irradiation and at the end of storage. The results demonstrated that flavanones such as naringin and narirutin and terpenoids such as limonene, 17-β-d-glycopyranoside, β-carotene and lycopene have been enhanced during storage for 35 days. It was observed that concentrations of

these biomolecules had significantly enhanced at low dose irradiation of the grapes vis-à-vis those at high doses (Patil et al., 2004).

1.1.2.2 Enhanced total phenols, antioxidants and anthocyanin contents in three different varieties of Persian pistachio nuts

Three varieties of Persian pistachio nuts were exposed to γ-irradiation at doses 0, 1, 2 and 4 kGy. Total phenols, antioxidant property in terms of DPPH and FRAP and anthocyanin content of γ-irradiated nuts were determined post γ-irradiation. From the results obtained, it was evident that total phenolic content and antioxidant content had increased at doses 1 and 2 kGy. Although 1 kGy had no significant effect on anthocyanin content of *Kale-Ghouchi* (K) and *Akbari* (A) genotypes, it significantly increased the anthocyanin content in the *Ghazvini* (G) genotype. However, a high dose of 4 kGy was needed to obtain a significant increase in anthocyanin contents of K and G genotypes (Akbari et al., 2018).

1.1.2.3 Enhanced proline, chlorophyll, total phenolic contents and antioxidant property in *Terminalia arjuna* Roxb

Terminalia arjuna, one of the potent medicinal plants for cardiac disease was subjected to γ-irradiation at doses of 0.025, 0.05, 0.1, 0.15, 0.2 kGy. An increase in proline content was observed with increase in dose. Chlorophyll content was observed highest at 0.1 kGy. Increased phenolic content and radical scavenging capacity was observed at 0.025 and 0.15 kGy (Akshatha et al., 2019).

1.1.2.4 Enhanced lutein content in corn (*Zea mays L.*) grits

Corn grits packaged in low density polyethylene (LDPE) were subjected to γ-irradiation in the dose range of 0.02-3 kGy and stored in an environmental chamber (23±2°C, 80% RH). From the analyzed data, it is evident that the highest dose of 3 kGy led to maximum enhancement in production of lutein (Pal 2018).

1.1.2.5 Enhanced lutein content in marigold (*Tagetes erecta* L.) flowers

Marigold flowers packaged in LDPE were subjected to γ-irradiation in the dose range of 0.02-2.5 kGy and stored in an environmental chamber (23±2°C, 80% RH). Here too, a high dose of 2.3 kGy resulted in enhanced production of lutein compared to the other doses (Pal and Bhattacharjee, 2016).

From the literature studies, it is evident that subjecting post-harvest produce to γ-irradiation, enhancement of bioactive compounds including antioxidants occurred. To the best of our knowledge, there is no literature report on enhancement of serotonin and melatonin in green plantains using this green technology.

1.2 Objectives

The specific objectives of the current investigation are:

i) To quantify L-tryptophan, serotonin and melatonin contents in plantains

ii) Enhancement (if any) of production of antioxidant biotherapeutic molecules, chiefly serotonin and/or melatonin in γ- irradiated plantain.

2. MATERIALS AND METHODS

2.1 Reagents and Samples

Fresh green plantains (*Musa paradisiaca*) of *desi* variety were procured from cultivators of Barasat, 24 Parganas North, West Bengal (22˚ 71′ N and 88˚ 51′ E, at about 13 m elevation above mean sea level, located in the eastern Gangetic plains of India) in September 2018 and were authenticated by West Bengal Food Processing and Horticulture Development Corporation Limited, Kolkata, India. The fruits were cultivated under tropical-temperate climate (24-32˚C, 75-85% RH) in sandy loamy soil of pH 7-8.

Specialty chemicals such as acetonitrile (99.9% pure, HPLC grade) and acetic acid of HPLC grade (99.8% pure) were purchased from M/s Merck, Darmstadt, Germany. L-tryptophan, serotonin and melatonin standards were purchased from M/s Sigma-Aldrich, Munich, Germany. For solid phase extraction, SPE tubes and dispersive 2ml universal kit were purchased from Agilent Technologies, Wilmington, USA. Liquid nitrogen was procured from Indian Refrigeration Stores, Kolkata, West Bengal. All reagents were of AR grade unless specified.

Γ-irradiation has been carried out at the Food Irradiation Laboratory of National Instruments Ltd. (NIL) campus, Jadavpur University, Kolkata, in GC-5000 unit of Board of Radiation and Isotope Technology, (BRIT, India) using ^{60}Co as the γ-radiation source. The surface radiation of GC-5000 was measured with a Minirad survey meter (Pulsecho, India) and was within safe operational limits (< 2 m rad). The dose rate during irradiation was 3.062 kGy h^{-1}. This was measured automatically by the unit and displayed on the front panel. The calibration and dose rate certification of the GC-5000 gamma chamber were conducted by BRIT in their laboratory using a standard Fricke's Dosimeter and the uncertainty in the measured dose was recorded at ± 5%.

2.2 Details of γ-irradiation facility employed in this study

In this study, GC-5000 of BRIT, Mumbai with ^{60}Co source was employed for irradiation of plantain. The schematic diagram of the unit has been shown in Figure 5. The dimensions of this irradiation unit are 1250 mm x 1065 mm x 1500 mm and its weight is 5.6 tonnes. Inside the γ-

chamber, the source is placed within a cylindrical cage, deep within a pit of dimensions 350 mm diameter x 700 mm. The entire unit has adequate heavy metal shielding with Pb and steel to restrict surface radiation within the maximum permissible limit of 2 mrad. During irradiation, effective dose rate of 3.062 kGy/h is obtained at the center of the sample chamber. The sample to be irradiated is placed inside the sample loading chamber (172 mm x 205 mm) located in a vertical drawer inside Pb shielding. There is a motorized drive to place the sample at the center of the radiation field. There is also provision for rotation of the sample up to a speed to 60 rpm for uniform dose distribution within the sample. The minimum duration that the sample must be exposed to the γ-source is 23 sec. The unit could be used in both auto and manual mode (Ghosh and Bhattacharjee, 2016). A schematic diagram of the GC-5000 unit is represented in Figure 5.

2.3 Methods

2.3.1 Optimization of γ-irradiation conditions for enhancing L-tryptophan, serotonin and melatonin contents in green plantain

2.3.1.1 Sample preparation for γ-irradiation of green plantains

Fresh green plantains were procured and stored at 23±2°C, 80% RH prior to irradiation. At the time of γ-irradiation, plantains were separated from their bunch, stalk was sized and they were placed in an amber colored beaker for γ-irradiation. The beaker was placed at the geometric center of the sample chamber and locked using the 'removable cover' of the unit. The fruits were γ-irradiated in a selected dose range.

2.3.1.2 Optimization of γ-irradiation process parameters for enhancing L-tryptophan, serotonin and melatonin contents in green plantain

In the preliminary study, green plantains were subjected to γ-irradiation in a dose range of 0.1-1 kGy. From the said study, it was observed that contents of L-tryptophan, serotonin and melatonin were relatively higher in γ-irradiated plantains for doses of 0.1 and 0.6 kGy. To enhance the contents of these aforesaid biomolecules in green plantains, a new study was designed introducing rotation during irradiation and polystyrene packaging inside the beaker for cushioning the banana samples. The GC-5000 unit was operated in rotation/stirring mode, which guaranteed uniform distribution of dose for each sample (IAEA, 2002). The free space in the

beaker was filled with polystyrene sheets to ensure proper cushioning of the plantains inside the beaker. Polystyrene was used for packing as effect of γ-irradiation at high dose on polystyrene is found negligible and it has been confirmed as the ideal polymer for food packaging during irradiation (Lijun Wang, 2008). The temperature of the ^{60}Co chamber during irradiation was 23 ± 2°C.

Plantains were subjected to γ-irradiation at different combinations of operational parameters, viz. with and without rotation mode and with and without polystyrene. Estimation of L-tryptophan, serotonin and melatonin contents in the γ-irradiated plantains was conducted to obtain the irradiation parameters that yielded relatively higher amounts of the three biomolecules. It was found that the plantains γ-irradiated at 0.1 and 0.6 kGy under rotation mode and cushioned with polystyrene sheets yielded satisfactory results. From these findings, a new experiment was designed, where plantains were subjected to γ-irradiation in the dose range of 0.02-1 kGy under the pre-optimized experimental conditions. The said dose range was selected since at a dose beyond 1 kGy, browning was observed in the γ-irradiated plantains within a day.

A total of 7 sample sets of green plantains were subjected to irradiation and one sample set served as the experimental control (viz. non-irradiated). Post irradiation, the plantains were stored at 23 ± 2°C, 80% RH for a total period of 25 days. Each set comprised of 75 samples. Each day, 3 samples were withdrawn from each set and subjected to solid phase extraction for extraction and quantitative analysis (discussed below) of the three biomolecules.

2.3.2 Solid phase extraction (SPE) of green plantains to extract L-tryptophan, serotonin and melatonin

2.3.2.1 Sample preparation for SPE

For extraction of L-tryptophan, serotonin and melatonin from green plantains (whole fruit was used along with their peel, except the stalk and end black part), the fruits were diced and cryo-ground to powder in accordance with the method described by Okazaki et al. (2009), with few modifications. 20 g of diced sample were taken in a polystyrene box; 160 ml of liquid nitrogen was added from a 35 L canister and allowed to freeze. The frozen sample was ground in a mixer

grinder (Philips Mixer Grinder, HL 1618, Philips India Limited, Chennai, India). This cryo-ground sample was used for SPE.

2.3.2.2 SPE of the cryo-ground green plantain

SPE of the cryo-ground plantain sample was conducted in accordance with the method described by Michelangilo and Stevan (2003), with few modifications. 15 g cryo-ground sample was taken in a clean 50 ml centrifuge tube where 15 ml of 1% acetic acid in acetonitrile solution and a SPE AOAC packet were added. The mixture was vortexed thoroughly. The tube was then centrifuged at 1500 x g for 1 min. One ml filtrate was withdrawn and added to dispersive-SPE (dSPE) sample cleanup tube containing specific chemicals and thoroughly mixed. dSPE sample cleanup tubes were then centrifuged at 1207 x g for 5 min. The supernatant i.e., the extracted sample was filtered using micro syringe filter (nylon 0.2 µm) and used for further analyses.

2.3.3 Quantification of L-tryptophan, serotonin and melatonin by high performance liquid chromatography (HPLC)

Solid phase extracted plantain extract was then subjected to analysis of L-tryptophan, serotonin and melatonin by HPLC in accordance with the method reported by Chakraborty and Bhattacharjee (2018), with slight modifications. 20 µl of the filtered extract was injected into JASCO HPLC (LC-Net-2/ADC, PU-4180 HPLC pump, DG- 4000-04 degasser, MD-4015 detector) system. The pump was operated in SPG mode with a pressure range of 0-50MPa. A C18 reversed phase column (l = 250 mm and I.D = 4.6 mm) was used. HPLC grade methanol and 1% HPLC grade acetic acid in distilled water were used as mobile phase solvents in gradient mode at a flow rate of 1 ml/min. The eluents were continuously monitored in a PDA detector having D2 lamp at 280 nm in accordance with the method reported by Huang and Mazza (2011). Peak identification was based on the retention time of Sigma standards L-tryptophan, serotonin and melatonin.

2.4 Statistical analysis

All extractions and analysis were performed for three independent HPLC runs of three samples withdrawn from each irradiated sample set (described in 'methods' section). Duncan's multiple range tests were carried out to analyze the effects of different irradiation dose with storage time. The differences among the mean values were determined at a confidence level of $P< 0.05$ in STATISTICA 8.0 (Statsoft, Oklahoma, USA).

3. RESULTS AND DISCUSSION

3.1 L-tryptophan, serotonin and melatonin contents in γ-irradiated green plantain

Figure 6 represents the chromatogram of solid phase extracted plantain extract containing L-tryptophan, serotonin and melatonin. Retention time of L-tryptophan was ~ 13.5 min, serotonin ~ 8.5 min and melatonin ~ 25min. Plantains subjected to γ-irradiation at doses of 0.1 and 0.6 kGy under rotation inside polystyrene cushion had relatively higher contents of L-tryptophan, serotonin and melatonin (shown in Figures 7 and 8). Rotation aided in uniform distribution of gamma rays and cushioning with polystyrene restricted movement of plantains inside the sample chamber (beaker) by minimizing the free space, in consonance with our earlier finding on increased lutein recovery from irradiated marigold flowers (Pal and Bhattacharjee, 2016).

L-tryptophan, serotonin and melatonin contents in γ-irradiated (in dose range 0.02-1 kGy) green plantain were quantified with progression of senescence on each day for the entire storage period of 25 days. Highest content of L-tryptophan was observed on day 1 in plantains γ-irradiated at 0.02 kGy, followed by the same in plantains γ-irradiated at 1 kGy. Highest content of serotonin was observed in 1 kGy-irradiated plantains on day 10 followed by that in 0.1 kGy-irradiated plantains on day 12. Melatonin content was found to be the highest in 0.04 kGy-irradiated plantains on day 16 followed by that in 0.6 kGy-irradiated plantains on day 12. However, plantains γ-irradiated at 0.6 kGy on day 10 of storage had significantly ($p < 0.05$) higher contents of L-tryptophan, serotonin and melatonin. Changes in L-tryptophan, serotonin and melatonin contents with storage have been shown in Figures 9, 10 and 11, respectively; and changes in the same in 0.6 kGy γ-irradiated plantains during storage is shown in Figure 12.

Γ-irradiated plantains possessed highest content of L-tryptophan (134.92 µg/g D.W) in their initial stage of senescence, which decreased gradually (9.688 µg/g D.W) with storage. 0.02 kGy γ-irradiated plantains had highest content of L-tryptophan on day 1, which decreased by 92.81% on day 16. Serotonin content in plantains increased and attained maximum value on day 10 which decreased steadily thereafter. It is evident that plantains γ-irradiated at 0.1 kGy had 89.44% increase in serotonin content on day 12, which decreased by 82.5% on day 16. However, melatonin content steadily increased throughout the storage period.

Bioconversion of serotonin to melatonin enhances content of the latter at the end stage of senescence period with concomitant reduction in serotonin content. Plantains irradiated at 0.04

kGy exhibited 82.46% increase in melatonin content on day 16 with simultaneous 86 % decrease in serotonin content. This is in harmony with the biosynthetic pathway of melatonin production in plants (Bioa and Oinghna, 2016). However, to the best of our knowledge, there is no literature on biosynthesis of melatonin through the established pathway in γ-irradiated fruits and vegetables to support our findings.

4. CONCLUSION

This work reports for the first time on the enhancement of contents of serotonin and melatonin in green plantains by γ-irradiation. From the results obtained, it was observed that L-tryptophan level was highest in the γ-irradiated plantains during the initial stage (day 1) of senescence and decreased thereafter. Serotonin content was observed to be highest on day 10 of the senescence period and had decreased significantly afterward; however, melatonin content in the same was highest at the end stage (day 16) of senescence period. These results are indicative of the conversion of L-tryptophan to melatonin via serotonin in concordance to reported biosynthesis pathway of serotonin and melatonin form precursor L-tryptophan in plants. Plantains γ-irradiated at 0.6 kGy on day 10 had relatively higher contents of L-tryptophan, serotonin and melatonin, among plantains γ-irradiated in the dose range of 0.02-1 kGy and significantly higher than their non-irradiated counterparts. Thus γ-irradiated plantains can be harnessed as potential sources of these important biotherapeutic molecules.

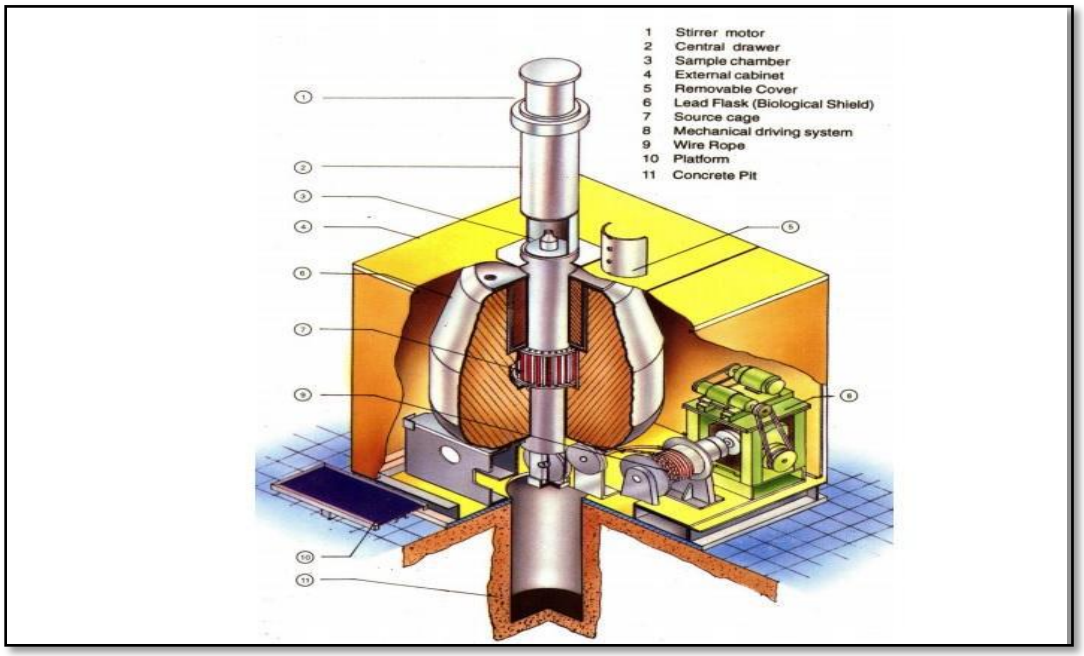

Figure 5: Schematic diagram of γ-irradiation (GC-5000) chamber

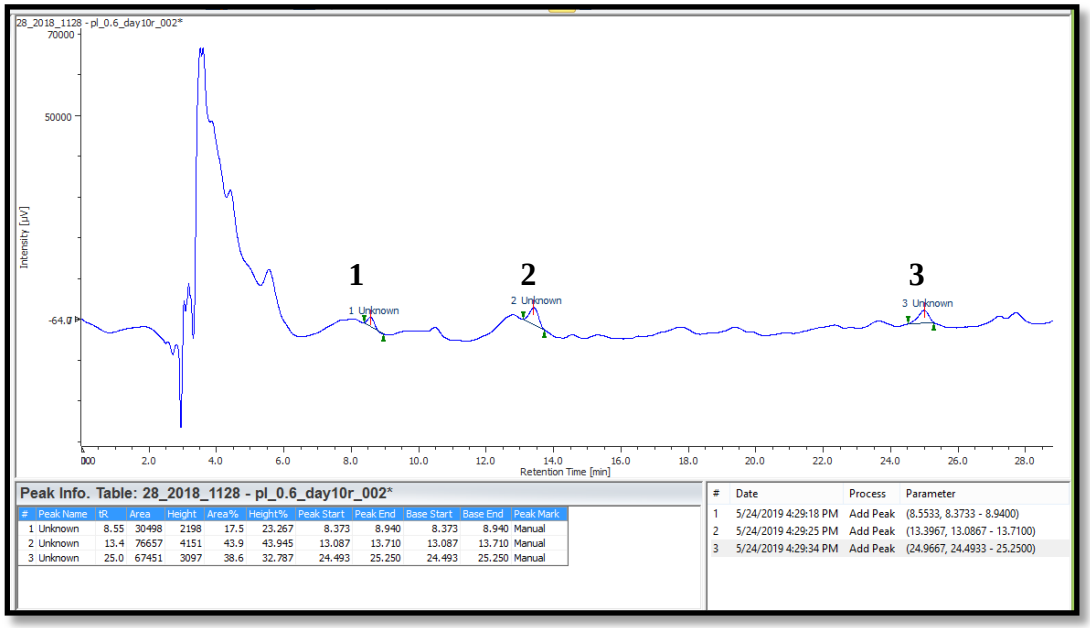

Figure 6: HPLC chromatogram of green plantain extract containing L-tryptophan (2), serotonin (1) and mela1tonin (3)

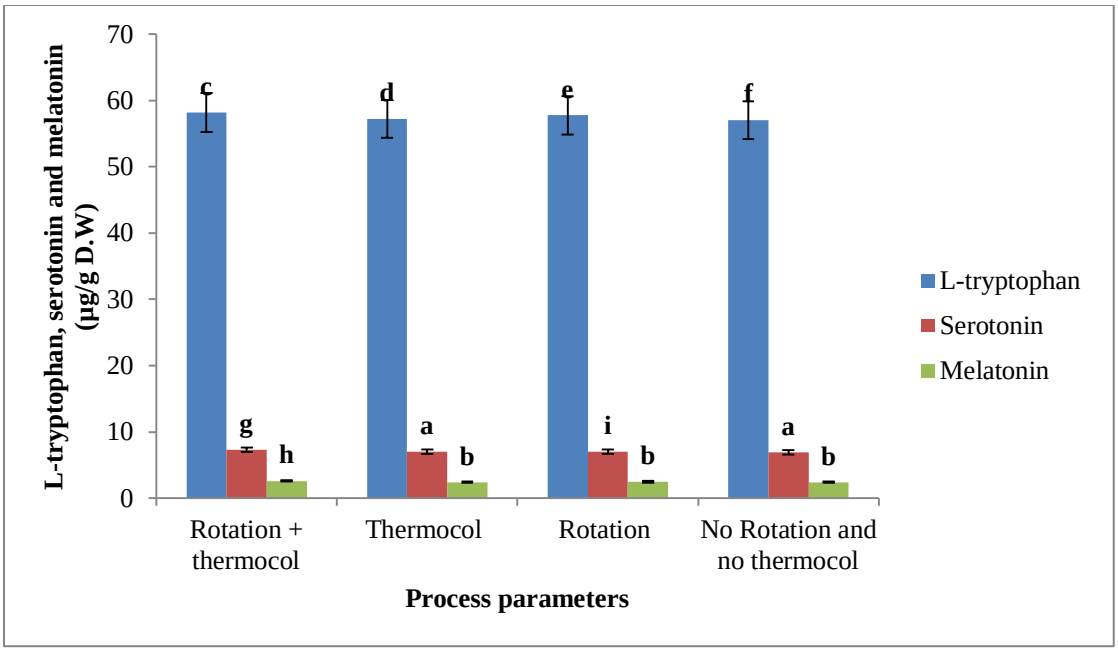

Figure 7: Changes in L-tryptophan serotonin and melatonin contents with process parameters in 0.1 kGy γ-irradiated plantains. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p < 0.05$

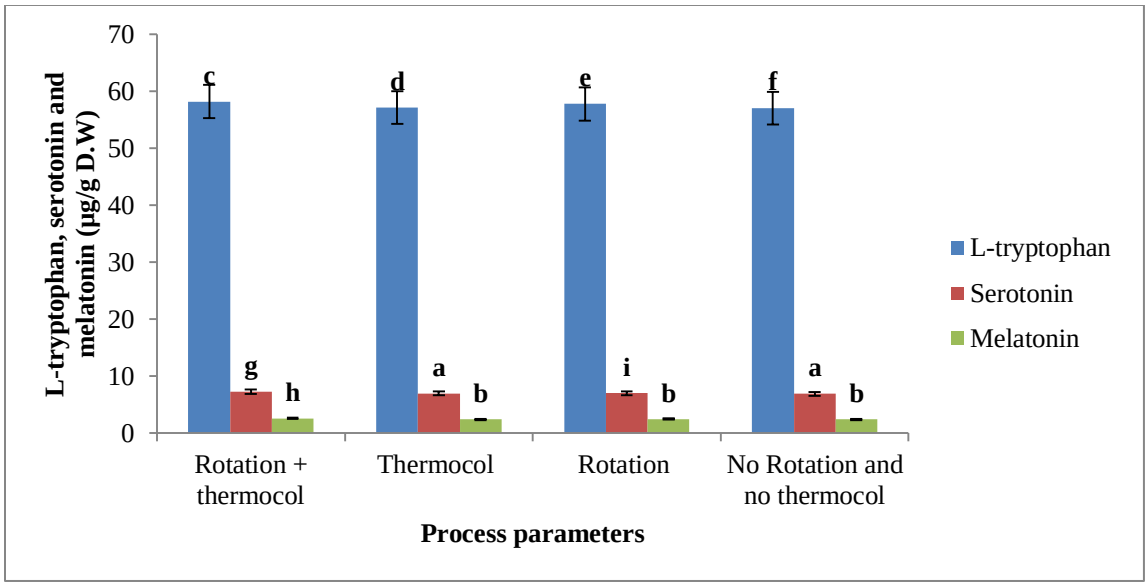

Figure 8: Changes in L-tryptophan, serotonin and melatonin contents with process parameters in 0.6 kGy γ-irradiated plantains. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar at $p < 0.05$ alphabets indicate that the mean values belong to different subsets

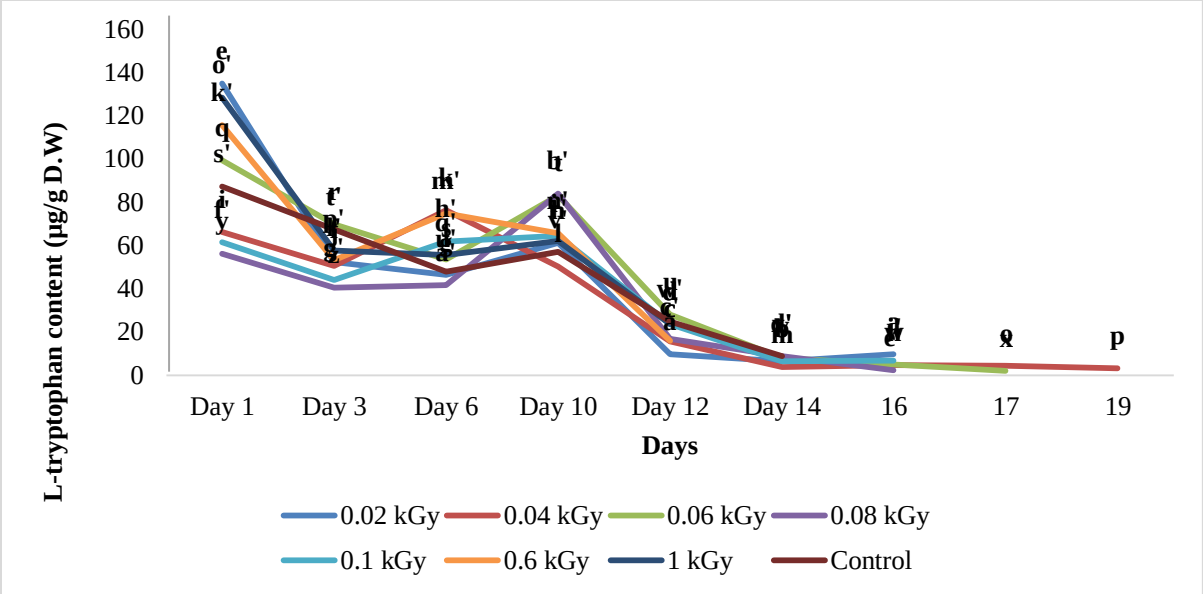

Figure 9: Changes in L-tryptophan, contents in control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

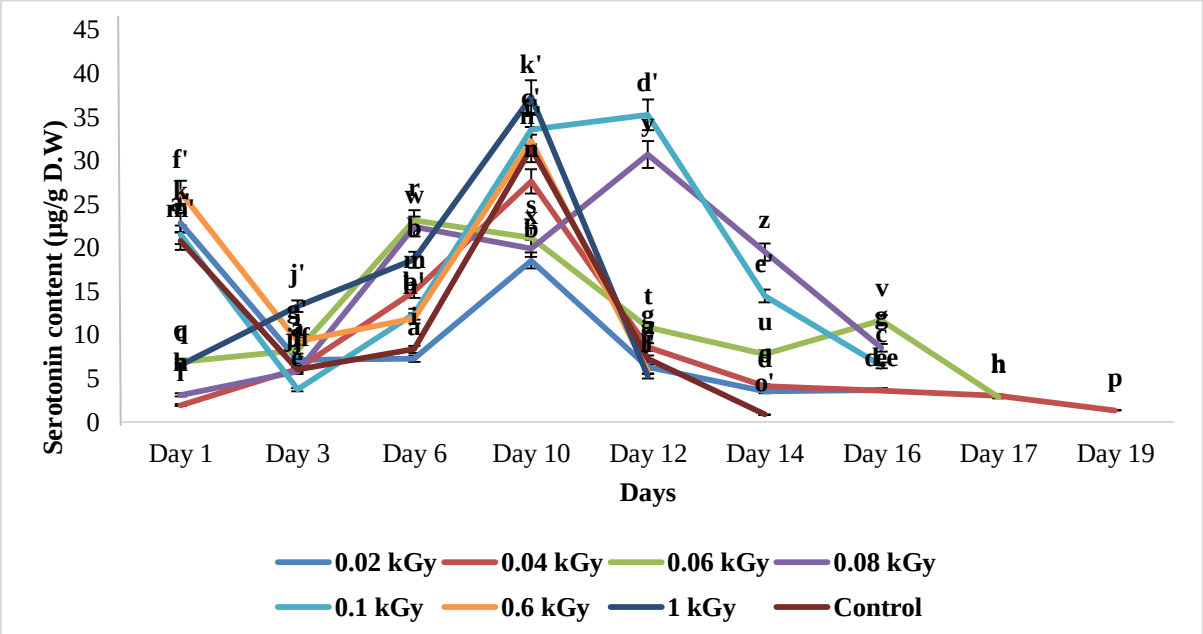

Figure 10: Changes in serotonin, contents in control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

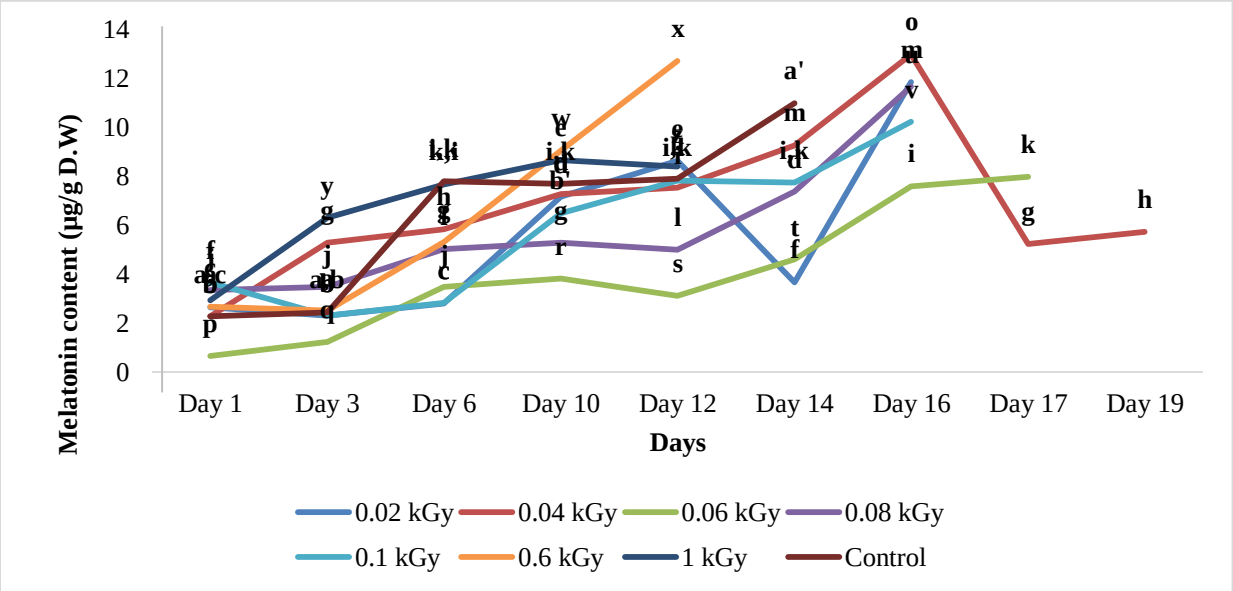

Figure 11: Changes in melatonin, contents in control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

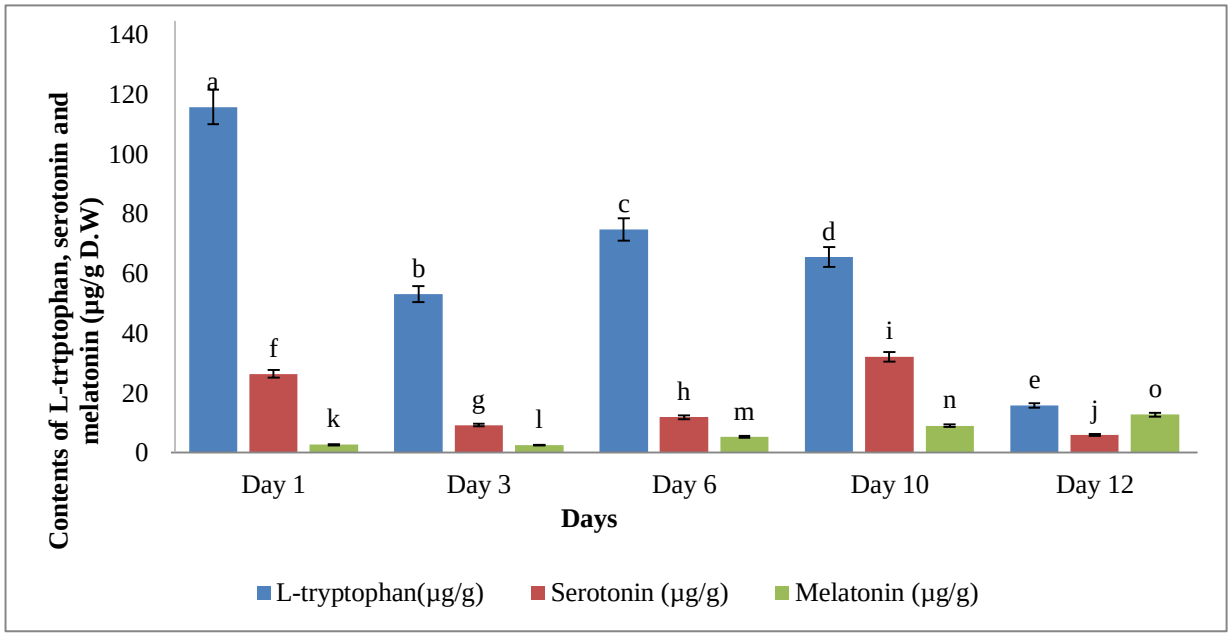

Figure 12: Changes in L-tryptophan, serotonin and melatonin, contents in 0.6 kGy γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

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1.1 INTRODUCTION

In the preceding chapter, quantification of bioactive molecules L-tryptophan, serotonin and melatonin contents in plantains during senescence when stored at 23±2°C, RH 80% post γ-irradiated in the dose range of 0.02-1 kGy have been reported. Since the aforesaid biomolecules are present in very low amounts (in µg to ng levels) in plantains, it is necessary to employ reliable methods for complete recovery and analyses of the same in the extracts. These biomolecules have been extracted from the γ-irradiated plantains employing SPE and were quantified in the extracts using HPLC-PDA. From the results obtained, it was observed that L-tryptophan level was highest in the γ-irradiated plantains during the initial stage (day 1) of senescence and decreased thereafter. Serotonin content was observed to be highest on day 10 of the senescence period and had decreased significantly afterward; however, melatonin content in the same was highest at the end stage (day 16) of senescence period. These results are indicative of the conversion of L-tryptophan to melatonin via serotonin in concordance to reported biosynthesis pathway of serotonin and melatonin form precursor L-tryptophan in plants.

In the present study, analytical parameters of HPLC were optimized to simultaneously quantify L-tryptophan, melatonin and serotonin in SPE extract of γ-irradiated plantains. Simultaneous quantification of more than one analyte using HPLC has been reported in only a few studies. Tryptophan, tryptophan methyl ester and melatonin contents were quantified from red wine by Fracassetti et al. (2019). Simultaneous quantification of 13 phenolic bioactive compounds from guava has been reported by Santos et al. (2017). To the best of our knowledge, no study has been reported on simultaneous quantification of the L-tryptophan, melatonin and serotonin in HPLC analyses. In order to validate the HPLC analyses, it is necessary to assess the mass transfer of the analytes in HPLC packed bed in terms of dimensionless numbers namely Reynolds (Re), Schmidt (Sc) number and Peclet (Pe). The data generated from the present investigation would be useful in validating the HPLC method used for simultaneous quantification of low amounts of L-tryptophan, serotonin and melatonin from similar matrices.

1.1 REVIEW OF LITERATURE

1.1.1 L-tryptophan, serotonin, melatonin quantification by HPLC

Quantification of L-tryptophan, serotonin and melatonin contents depend majorly on two parameters namely their extraction procedure from botanicals and on the method of analysis used to quantify the same in the extract. Inappropriate method of extraction of the bioactive molecules will lead to inaccurate quantification of the same. Additionally, inaccurate quantification may also be due to instability of the extract containing melatonin and serotonin (Huang and Mazza, 2011). Analysis of the bioactive molecules can be conducted employing relatively new and rapid technologies such as by HPLC equipped with photodiode array (PDA), fluorescence detector (FD), electrochemical detector (ECD) and HPLC coupled to mass spectrometry (LC-MS). Depending on the type of detector, sensitivity, predictability, reproducibility of the data varies. There are several reports on analyses of L-tryptophan (Fracassetti et al., 2019 and Islam et al., 2015), serotonin (Hosseinian et al., 2008 and Lavizzari et al., 2006) and melatonin (Burkhardt et al., 2001; Chen et al., 2003; Pape and L¨uning, 2006; Hosseinian et al., 2008) using HPLC with FD, ECD and UV detectors. Literature study on the determination of L-tryptophan, serotonin and/or melatonin contents from various post-harvest produces by HPLC with different detectors is tabulated in Table 1.

1.1.2 LOD and LOQ

In general, LOD is considered as the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test (Shrivastava and Gupta, 2011).

1.1.2.1 LOD and LOQ of L-tryptophan, tryptamine and serotonin

LOD and LOQ of L-tryptophan, tryptamine and serotonin have been reported in selected fruits namely kiwi, banana, pineapple, avocado and in vegetables such as spinach, lettuce and cabbage when analyzed by HPLC with FD detector (Islam et al., 2015).

1.1.2.2 LOD and LOQ of 13 phenolic bioactive compounds in guava

LOD and LOQ of 13 phenolic bioactive compounds in guava namely, ferulic, gallic, caffeic, chlorogenic, transcinnamic, vanilic, elagic, pcoumaric and syringic acids; quercetin; (+) catechin, rutin, kaempferol and (±)-6-hydroxy-2, 5, 7, 8-tetramethylchromane 2-carboxylic acid when analyzed by HPLC-PDA have been reported by Santos et al. (2017).

1.1.3 Mass transfer modeling of free lutein (in marigold flower extract) in analytical HPLC column using Langmuir adsorption model

Mass transfer of free lutein derived from marigold in a chromatographic process was mathematically modeled using Langmuir adsorption model. Adsorption isotherm of lutein in the HPLC column was determined from experimental adsorption study and the axial dispersion and mass transfer coefficient thus obtained were fitted into three mathematical models, namely ideal model, equilibrium-dispersive model, and transport model. From Langmuir isotherm, it was found the transport model best described the mass transfer behavior of free lutein in both linear and non-linear ranges of concentration (Clowutimon et al., 2018).

From the above literature study, it is evident that L-tryptophan, serotonin and melatonin can be extracted through various processes and can be analyzed by HPLC with various detectors. In the previous chapter, we have also reported on quantification of L-tryptophan, serotonin and melatonin (in SPE extract of plantains) employing HPLC-PDA. The aforesaid bioactive molecules were found to be present in very low amounts in the plantain extracts. To the best of our knowledge, there is no literature report on simultaneous quantification of L-tryptophan, serotonin and melatonin employing HPLC analysis. Therefore, in order to establish the HPLC analysis as a reliable method for simultaneous quantification of these bioactive molecules, it is necessary to validate the HPLC method by mass transfer modeling of L-tryptophan, serotonin and melatonin in the HPLC C-18 column, erstwhile not reported in literature.

1.2 Objectives

The specific objectives of the current investigation are:

i) To determine the LOD and the LOQ of the three biomolecules employing HPLC method of analysis (at pre-optimized parameters).

ii) To validate the aforesaid method of analysis based on the mass transfer study of the analytes in the packed bed of HPLC column (C18) using dimensionless numbers namely Re, Sc and Pe and to determine a correlation among them employing regression modeling.

2. MATERIALS AND METHODS

2.1 Materials

Materials used in this experiment are same as described in section 2.1 of Chapter 1.

2.2 Method

2.2.1 HPLC analysis to quantify L-tryptophan, serotonin and melatonin contents in SPE extract of plantains

Solid phase extracted plantain extract was subjected to quantification of L-tryptophan, serotonin and melatonin by HPLC-PDA in accordance with the method reported by Chakraborty and Bhattacharjee (2018), with modifications. By preliminary trials, flow rate of mobile phase and gradient mode elution programme of mobile phase (HPLC grade methanol and 1% HPLC grade acetic acid in distilled water were used as mobile phase; linear gradient elution was applied from 10% to 90% methanol within 30 min at a flow rate of 1 ml/min) were optimized. 20 µl of the filtered extract was injected into JASCO HPLC (LC-Net-2/ADC, PU-4180 HPLC pump, DG-4000-04 degasser, MD-4015 detector) system. The pump was operated in SPG mode with a pressure range of 0-50MPa. A C18 reversed phase column (l = 250 mm and I.D. = 4.6 mm) was used. The eluents were continuously monitored in a PDA detector having D2 lamp at 280 nm in accordance with the method reported by Huang and Mazza (2011). Peak identification was based on the retention time of Sigma standards L-tryptophan, serotonin and melatonin.

2.2.1.1 Determination of LOD and LOQ of L-tryptophan, serotonin and melatonin

Determination of LOD and LOQ were performed for HPLC method of analysis in accordance with the official guidelines (ICH 2005, Ribani et al., 2004). LOD and LOQ were estimated experimentally by injecting standard solutions of the three biomolecules diluted in methanol until, the signal-to-noise ratio for the standards reached a 3:1 ratio for LOD and 10:1 for LOQ (Zeraik and Yariwake, 2010). Three experimental runs were conducted for each concentration.

2.2.1.2 Mass transfer of L-tryptophan, serotonin and melatonin in packed bed C18 HPLC column

In previous investigations, our research team had successfully conducted mass transfer studies on supercritical carbon dioxide (SC-CO_2) extraction of bioactive components from several

botanicals, where the packed beds comprised of botanical matrices and the extracting solvent was SC-CO_2. In this study, we have employed the similar mass transfer approach in terms of dimensionless numbers, since HPLC column (C18) used in this study supposedly mimics a typical packed bed and the mobile phase used plays the role of an extracting solvent.

The nature of flow of the fluid i.e., the mobile phase along with SPE extract (containing L-tryptophan, serotonin and melatonin) through the stationary phase was determined by evaluating Re of the HPLC mobile phase.

$$Re = \frac{\rho V l}{\mu} \quad (1)$$

where, ρ = density of the fluid, V = velocity of the fluid, μ = viscosity of fluid, L = diameter of the column.

For determination of Re, the following parameters were used: diameter (L) of the column = 0.46 cm, density (ρ) of mobile phase i.e., HPLC grade methanol and HPLC grade 1% acetic acid = 0.9156 g/cc, velocity (V) of the mobile phase = 0.0963 cm/s and viscosity (μ) of the mobile phase = 0.0119 P. μ of 50% methanol at 20°C had been estimated using viscosities reported by Perry and Green (1997). ρ of aforesaid 50% methanol at 20°C have also been reported in this reference.

Sc is the ratio of the kinematic viscosity to the molecular diffusion coefficient. For evaluation of Sc, molecular diffusivities of the biomolecules namely L-tryptophan, serotonin and melatonin were calculated using Wilke-Chang equation (Paul and Bhattacharjee, 2018).

$$Sc = \frac{v}{D} \quad (2)$$

where, v = kinematic viscosity, D= molecular diffusivity

Pe is a mass transfer coefficient and is defined as the ratio of the rate of advection of a physical quantity by the flow to the rate of diffusion of the same quantity driven by an appropriate gradient. Eq. 3 is used for evaluation of Pe.

$$Pe = \frac{LV}{D} \quad (3)$$

2.3 Statistical analysis

Three independent HPLC runs were conducted using the SPE extract to evaluate Re, Sc and Pe. Regression analysis of mass transfer (of L-tryptophan, serotonin and melatonin in packed bed) data was conducted using STATISTICA 8.0 (Statsoft, Oklahoma, USA) to obtain an empirical correlation among Re, Sc, and Pe numbers.

3. RESULTS AND DISCUSSION

3.1 LOD and LOQ of L-tryptophan, serotonin and melatonin by optimized HPLC programing

The LOD values for L-tryptophan, serotonin and melatonin obtained by HPLC analyses were 1.16 µg/l, 0.12 µg/l and 0.21 µg/l, respectively. Obtained LOQ values for L-tryptophan, serotonin and melatonin were 1.88 µg/l, 0.89 µg/l and 0.51 µg/l, respectively.

3.2 Mass transfer of L-tryptophan, serotonin and melatonin in packed bed HPLC column

Re of mobile phase flowing through the stationary phase in HPLC was 3.4083 indicating laminar flow. This result was in concurrence with study reported by Berger et al. (2017), who reported that fluid flow is always laminar in HPLC analysis. Sc for L-tryptophan, serotonin and melatonin are in the range of 15-18. which indicates mass diffusion of biomolecules in the mobile phase. Additionally low Sc (<200) signified good mass diffusivity of analytes through laminar layer of the fluid (Camuffo, 2014). Pe for L-tryptophan, serotonin and melatonin were 2860.809, 2618.486 and 3090.468 respectively, high Pe indicates higher rate of advection in the column. These results indicated proper elution of the analytes from the HPLC column by the mobile phase (flowing at a constant flow rate of 1 ml/min) in HPLC and thus establishing reliability of the HPLC chromatogram (Figure 6 in Chapter 1). Mass diffusivities of the three biomolecules and the calculated Re, Sc and Pe are tabulated in Table 2.

For assessing mass transfer in a packed bed by correlating the above mentioned dimensionless numbers, Tan and Liou (1989) proposed the following relation (similar to that in case of SC-CO_2 extraction):

$$Pe=A.Re^{a}.Sc^{b} \quad (4)$$

Substituting the calculated Re, Sc and Pe numbers in the above equation and applying multiple regression analysis we obtained the following equation:

$$Ln\ (Pe/Sc^{0.98}) = a.lnRe + lnA \quad (5)$$

R^2 value of Eq. 5 was 0.999 and standard error obtained was 0.005 for each biomolecule. The mass transfer equations correlating the dimensionless numbers were found to be:

$$Pe = 52.77.Re^{1.020}.Sc^{0.98} \quad (6)$$

Eq. 6 shows the dependence of Pe on Re number with linear relation between $(Pe) / (Sc)^{0.98}$ and (Re) with $R^2 = 0.999$ and standard error = 0.005. High correlation coefficient of the empirical equation suggested that the biomolecules namely L-tryptophan, serotonin and melatonin had been successfully simultaneously quantified by the HPLC method developed by our research group, affirming the method to be robust and reliable. Therefore LOD and LOQ values of aforesaid biomolecules obtained from this method of analysis are correct and valid.

4. CONCLUSION

In the present investigation, L-tryptophan, serotonin, and melatonin in SPE extract of plantains were analyzed simultaneously using HPLC method of analysis. Owing to the low contents of the biomolecules in plantains and simultaneous quantification of these molecules by HPLC, mass transfer of the biomolecules and the mobile phase flowing through the packed bed were conducted in terms of Re, Sc, and Pe numbers. High correlation coefficient among the dimensionless numbers suggested the reliability of the HPLC method signifying the method to be robust. Thus, LOD and LOQ obtained for L-tryptophan, serotonin, and melatonin were correct and valid. This method of analysis employing HPLC can be used for quantification of L-tryptophan, serotonin and melatonin, extracted from other fruits and vegetables.

Table 1: HPLC methods for L-tryptophan, serotonin and melatonin determination

Compound	Plant product	Column	Mobile phase and elution	Detection	Reference
Melatonin	Edible plants seeds	RP-C18 column	0.1 mol/l potassium phosphate buffer, pH 4.5 – acetonitrile 80:20, 1 ml/min	ECD: initiated at 200 mV for channel 1; increased by 100 mV for each of the higher channels; resulted in 900 mV at channel 8	Manchester et al., 2000
Melatonin	Walnuts	RP-C18 column	0.1 mol/l potassium phosphate buffer, pH 4.5 – acetonitrile 80:20, 1 ml/min	ECD: initiated at 200 mV for channel 1; increased by 100 mV for each remaining channel	Reiter et al., 2005
Melatonin	Edible plants	Hypersil ODS (4.6 ×250 mm, 5 μm)	50 mmol/l Na_2HPO_4/H_3PO_4 buffer, pH 4.5 – methanol60:40, 1.0 ml/min	FD: excitation 280 nm emission 340 nm	Hattori et al., 1995

Compound	Plant product	Column	Mobile phase and elution	Detection	Reference
Melatonin	White lupine and barley seeds	Spherisorb-S5 ODS2(4.6 × 250 mm)	Water : acetonitrile60:40, 0.2 ml/min	FD: excitation 280 nm emission 350 nm	Hern´andez-Ruiz and Arnao 2008a
Serotonin	Potato fruits, leaves and tubers	LiChrospher 100 RP-C18	0.01 mol/l phosphate buffer, pH 2.8 – acetonitrile 0 –30 min, 5%–25% acetonitrile;1.5ml/min	FD: excitation 295 nm emission 330 nm	Engstrom et al., 1992
Serotonin	"Prata" banana	μ Bondapak C18 (3.9 × 300mm); guard column	0.01 mol/l phosphate buffer, pH 2.8 – acetonitrile 0 –30 min, 5%–25% acetonitrile; 1.5 ml/min	FD: excitation 295 nm emission 330 nm	Engstrom et al., 1992
Serotonin	Pepper leaf, stem, fruit, flower and root	Wakosil II 5C18HG (4.6 × 150 mm)	0.3% trifluoroacetic acid – methanol 95:5, 0.8 ml/min	UV 280 nm	Kang and Back, 2006
Serotonin	Tomato, cherry tomato, hot pepper, Chinese cabbage, Paprika	Atlantis C18 (3.9 × 150 mm)	0.3% trifluoroacetic acid – methanol 95:5, 0.8 ml/min	UV 280 nm	Ly et al., 2008

Compound	Plant product	Column	Mobile phase and elution	Detection	Reference
Melatonin	Edible plants seeds	RP-C18 column	0.1 mol/l potassium phosphate buffer, pH 4.5 – acetonitrile 80:20, 1 ml/min	ECD: initiated at 200 mV for channel 1; increased by 100 mV for each of the higher channels; resulted in 900 mV at channel 8	Manchester et al., 2000
Melatonin	Walnuts	RP-C18 column	0.1 mol/l potassium phosphate buffer, pH 4.5 – acetonitrile 80:20, 1 ml/min	ECD: initiated at 200 mV for channel 1; increased by 100 mV for eachremaining channel	Reiter et al., 2005
Melatonin	Edible plants	Hypersil ODS (4.6 ×250 mm, 5 μm)	50 mmol/l Na_2HPO_4/H_3PO_4 buffer, pH 4.5 – methanol 60:40, 1.0 ml/min	FD: excitation 280 nm emission 340 nm	Hattori et al., 1995
Melatonin	White lupine and barley seeds	Spherisorb-S5 ODS2 (4.6 × 250 mm)	Water : acetonitrile 60:40, 0.2 ml/min	FD: excitation 280 nm emission 350 nm	Hern´andez-Ruiz and Arnao 2008a
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Compound	Plant product	Column	Mobile phase and elution	Detection	Reference
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Serotonin	Pepper leaf, stem, fruit, flower and root	Wakosil II 5C18HG (4.6 × 150 mm)	0.3% trifluoroacetic acid – methanol 95:5, 0.8 ml/min	UV 280 nm	Kang and Back, 2006
Serotonin	Tomato, cherry tomato, hot pepper, Chinese cabbage, paprika, green	Atlantis C18 (3.9 × 150 mm)	0.3% trifluoroacetic acid – methanol 95:5, 0.8 ml/min	UV 280 nm	Ly et al., 2008
Melatonin	Chinese licorice seed, root, leaf and stem tissues	Phenomenex Hypersil C18 (4.6 × 100 mm, 3.0 µm) C18 guard column (4 × 3 mm)	0.1 mol/l Na_2HPO_4 acetonitrile 65:35, 1 ml/min	UV/VIS PAD	Afreen et al., 2006
Melatonin, Serotonin	Purple wheat	C18 (3 × 150 mm I.D., 5 µm)	Solvent A: 0.1% acetic acid in double-deionized water solvent B: 0.1% acetic acid in acetonitrile. 0–5 min, 10% solvent B; 5–10 min, 10%–40% solvent B; 10–40 min, 40% solvent B; 41–50 min, 10% solvent B. 0.5 ml/min	UV 280 nm	Hosseinian et al., 2008

Compound	Plant product	Column	Mobile phase and elution	Detection	Reference
Serotonin, L-tryptophan and tryptamine	Kiwi, banana, pineapple, mikan	C18 (4.x650 mm,5 μm)	10mM $HCOONH_4$, pH3.4;515min, linear gradient of 0–25% acetonitrile in 10mM $HCOONH_4$, pH 3.4, 1 ml/min.	FD: excitation 300nm emission 335 nm	Islam et al., 2015
L-tryptophan, tryptophan methyl ester and melatonin	Red wine	C18 (100 x 3 mm, 2.7 _m particle size)	0.1% formic acid (v/v), and methanol acidified with 0.1% formic acid (v/v), 0.5ml/min.	HPLC-MS and HPLC-FD	Fracassetti et al., 2019
Melatonin and Serotonin	Wheat	C18 (1.0 × 100 mm, 1.7 μm)	0.1% formic acid; methanol 0–8min, 13%–24% methanol; 8–22 min, 24%–100% methanol; 22–24 min, 100% methanol. 0.2 ml/min	MS- Quattro Micro API MS ESI source negative ion Mode	Hosseinian et al., 2008
Melatonin	Sour cherry cultivars Montmorency Balaton	Gemini C18 (2 × 150 mm I.D., 5 μm)	0.5% formic acid and methanol 0.4 ml/min	MS- LCT MS ESI source positive ion mode SRM mode	Kirakosyan et al., 2009

Table 2: Diffusivities of L-tryptophan, serotonin and melatonin in mobile phase and the calculated dimensionless numbers

Biomolecule	Mass diffusivity (cm^2/s)[a]	Reynolds number (Re)	Schmidt number (Sc)	Peclet number (Pe)
L-tryptophan	0.0008	3.4808	16.4044	2860.809
Serotonin	0.0007	3.4808	17.7213	3090.468
Melatonin	0.0009	3.4808	15.0149	2618.486

[a]Calculated using Wilkie–Chang equation

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1. Introduction

In the preceding Chapters 1 and 2, it has been reported that γ-irradiation treatment of plantains resulted in enhanced synthesis of L-tryptophan, serotonin and melatonin at varying levels during different stages of senescence of the plantains when stored at 23±2°C, RH 80%. The plantains irradiated at 0.6 kGy had relatively higher contents of the aforesaid biomolecules on day 10. These biomolecules in plantains were analyzed and quantified simultaneously by well validated and robust HPLC method of analysis (as elaborated in Chapter 2).

Despite being a rich source of the said biomolecules, plantains reportedly have high post-harvest losses (FAO, 1978). Owing to the perishable nature of the plantains, several preservation treatments such as modified atmosphere storage (Sanchez-Nieva et al., 1970 and Hernandez, 1973), use of ethylene absorbent (Ndubizu, 1976 and Olorunda, 1976), application of surface coating agent and dehydro-freezing (Olorunda, 1988) have been applied to the fruits to delay their ripening and to subsequently increase their shelf-life. However, the aforesaid methods are associated with certain limitations such as uneven color development, development of off-flavor and chilling injury (Aina, 1999). Additionally, some of these methods of preservation (such as atmosphere storage and dehydrofreezing) are very expensive. Earlier work conducted by our research team (Pal and Bhattacharjee, 2016) established that γ-irradiation not only enhanced production of lutein in γ-irradiated marigold flowers but also extended their shelf-life (lead of 5 days) as well, forgoing the above-mentioned limitations of other known preservation methods. This finding prompted us to investigate on extension of shelf life of the plantains employing the green technology of γ-irradiation.

1.1 Review of literature

1.1.1 Enhanced shelf-lives of fruits and vegetables by γ-irradiation

Γ-irradiation is reportedly known to enhance shelf-lives of several fruits and vegetables such as tomato, strawberry, pineapple, cucumber and cabbage. Few effects of γ-irradiation on fruits and vegetables have been enumerated in the following section.

1.1.1.1 Tomato

In tomatoes (*Solanum lycopersicum*) γ-irradiated in the range of 0.5-1 kGy, dose of 1 kGy resulted in maximum enhancement of shelf-life (with a lead of 11 days vis-à-vis its non-irradiated counterpart) of the tomatoes without compromising their sensory and nutritional values (Munir et al., 2018).

1.1.1.2 Strawberry

Effects of γ-irradiation at 1 kGy on quality of strawberry (*Fragaria ananassa*) stored at 4°C have been reported by Jouki and Khazaei (2013). Several quality parameters such as texture, microbial load and sensory parameters were assessed in the γ-irradiated strawberries during a storage period of 21 days. Irradiated strawberries maintained their texture and overall appearance better than their non-irradiated counterparts throughout the storage period. Irradiation increased shelf-life of the strawberries from 14 days to 21 days, without any microbial contamination.

1.1.1.3 Minimally processed cabbage and cucumber

In a study conducted by Khattak et al. (2004), minimally processed cabbages (*Brassica oleracea*) and cucumbers were γ-irradiated (*Cucumis sativus*) and stored at 5°C for 2 weeks. Cucumber γ-irradiated at 3 kGy was found to soften immediately after irradiation while texture of 0.5-2.5 kGy irradiated cucumber was acceptable sensorically. The γ-irradiation treatment had no effect on the texture and sensory scores of cabbage.

1.1.1.4 Pineapple

Pineapples (*Ananas comosus* L.) were subjected to γ-irradiation in a dose range of 0.05-0.30 kGy (Damayanti et al., 1992) and stored at room temperature (25-28°C) as well as at controlled temperature (11-13°C). From the report it was evident that γ-irradiated pineapples, particularly at doses > 0.25 kGy, had browning of the shin and softening of tissues. However, pineapple γ-irradiated at 0.15 kGy (stored at 25 to 28°C) retained their characteristic textures and among all the doses studied, irradiation at the dose range of 0.05 to 0.25 kGy (stored at 11 to 13°C) reportedly reduced maximum postharvest loss in the pineapples, implying maximum extension of their shelf life.

1.1.1.5 Banana

In a study reported by Zaman et al. (2007), bananas (*Musa sapientum*) have been treated at 0.3, 0.4 and 0.5 kGy for 5 min, respectively and stored under ambient conditions (25±2°C, 80±5% RH). From the sensory scores of the bananas, it was evident that shelf-life of γ-irradiated bananas has been extended by 20 days.

1.1.1.6 Plantains

Delay in ripening and consequent enhancement of shelf-life has been reported in the γ-irradiated plantains in dose range of 0.02-0.04 kGy (Thomas and Nair, 1971). The optimal radiation dose for inhibition of ripening and the maximum dose the plantains can tolerate without exhibiting phytotoxicity or radiation-induced injury, seem to differ among cultivators and even for the same cultivators grown in different geographic areas. However, irrespective of plantains grown in different locations, doses exceeding 0.5 kGy invariably resulted in browning or blackening of the skin in the fruits.

1.1.2 Physiology and biochemistry of plantains with ripening

During ripening, plantains experience rapid increase in respiration accompanied by marked changes in color (from green to yellow due to chlorophyll breakdown and carotenoid formation), texture (softening due to modifications in pectic acids), aroma and flavor (due to synthesis of typical volatile aromatic constituents and/or modifications in sugar: acid ratio), sweetness (due to increase in soluble sugars by hydrolysis of reserve polysaccharides such as starches) and the rate of ethylene development (Molins, 2001). These changes in plantains can be delayed by subjecting them to γ-irradiation (Molins, 2001).

From the above literature reports, it is established that γ-irradiation treatment can successfully extend shelf-lives of fruits and vegetables. However, the effective dose required for extension of shelf-lives varied with fruits and vegetables; hence optimization of γ-irradiation dose is essential. Additionally, physicochemical parameters of fruits and vegetables such as color, texture and sugar content reportedly changed during the above storage studies. Therefore the aforesaid parameters have been selected in this study to ascertain shelf-life of γ-irradiated plantains. To the best of our knowledge, there is no literature report on enhancement of shelf-life of green

plantains using this green technology in terms of sensory attributes, proximate analysis, physicochemical and phytochemical properties of plantains during storage.

1.2 Objective

The specific objective of the current investigation is to optimize the dose of γ-irradiation for - extending shelf-life of plantains by evaluating sensory attributes, proximate, physicochemical and phytochemical properties during storage.

2. MATERIALS AND METHODS

2.1 Materials

Plantains (*Musa paradisiaca L.*) were procured from Barasat area, Kolkata, from a registered farmer and authenticated by West Bengal Food Processing and Horticulture Development Corporation Limited, Kolkata. 1, 1- diphenyl-2-Picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) were procured from M/s Sigma-Aldrich, Munich, Germany, St. Louis, MO, USA. Folin Ciocalteu's reagent, trichloroacetic acid (TCA), ethylene diamine tetraacetic acid (EDTA), butylated hydroxyl toluene (BHT), ascorbic acid, 30% H_2O_2 and all the other chemicals were purchased from M/s Merck, Mumbai, India. All chemicals used in this work were of AR grade.

2.2 Methods

2.2.1 Γ-irradiation of green plantains

Sample preparation of green plantains for γ-irradiation has been conducted in accordance to the method mentioned in section 2.1 of Chapter 1. In the preliminary study, green plantains were subjected to γ-irradiation in a dose range of 0.1-1 kGy and it was observed that the plantains γ-irradiated at 0.1, 0.6 and 1 kGy had higher shelf lives vis-à-vis those γ-irradiated at other doses of 0.2, 0.4, 0.5, 0.8 kGy. In order to investigate the effects of γ-irradiation at doses <0.1 kGy, in the present study, plantains were subjected to γ-irradiation at doses *viz.* 0.02, 0.04, 0.06, 0.08 along with doses of 0.1, 0.6 and 1 kGy (selected from preliminary trials) under the pre-optimized experimental conditions (elaborated in Chapter 1). Post irradiation, the plantains were stored at 23±2°C, 80% RH for a total period of 25 days.

2.2.2 Sensory evaluation of γ-irradiated green plantains

Owing to the perishable nature of plantains, sensory evaluation of the γ-irradiated plantains during storage was determined. Sensory evaluation would allow identification of effective dose of irradiation required to achieve the highest shelf life of the plantains. The γ-irradiated plantains were therefore subjected to sensory evaluation by a semi-trained panel of university faculty and research scholars (15 men and 15 women) in the age group of 20-45 years. The panelists rated plantains on a 9-point hedonic scale (9 –like extremely and 1 – dislike extremely), in accordance with the method reported by Clark et al. (2013), with few modifications.

Sensory attributes investigated by the panel were color, odor, texture, shrinkage and overall appearance of plantains. Sensory evaluation of each set of plantain was carried out each day from day 1 (between 10 am and 12 pm) until the end of their respective shelf-lives, in a ventilated room under white light. The γ-irradiated plantain sets were arranged randomly and sensory evaluation was conducted in triplicate in each session. A rest time of 5 min was provided between evaluations of consecutive sets. The mean sensory score of the panel was considered for analysis and has been represented by radar plots. Shelf life of plantains was determined based on these sensory scores. Subsequently, to optimize the appropriate dose of irradiation, γ-irradiated plantain sets along with the control set were subjected to proximate, physicochemical and phytochemical analyses. The effects of storage time on the plantains were evaluated on day 0 (i.e., on the day of γ-irradiation) and successively on days 2, 6, 10, 14, 16 and 19 (the end of shelf-life of plantains having highest sensory scores). All assays were conducted on the above mentioned days apart from proximate analyses (such as ash content and fat content) which were conducted on days 0, 7 and 15 and physicochemical analyses (such as texture profile analysis and CIE color value determination) were conducted on days 0, 4, 8, 12 and 19. Each day, 3 samples were withdrawn from each set and subjected to storage study.

2.2.3 Proximate analysis of γ-irradiated green plantains

2.2.3.1 Analysis of moisture content

Moisture content of plantains were determined with 1 g diced sample using an IR moisture analyzer (Citizen MB 200 C) at 120°C in accordance with the method elaborated by Latorre et al. (2010).

2.2.3.2 Analysis of fat content

Total fat contents of the plantains were estimated gravimetrically by extraction with *n*-hexane in Soxhlet assembly according to AOAC method 920.39A (2000) and were calculated as % fat content.

2.2.3.3 Analysis of protein content

Protein contents of the plantains were determined by Lowry's method (Lowry et al., 1951), with few modifications. 5 g of plantains were homogenized in a pre-chilled mortar pestle with

potassium phosphate buffer (pH 7.4) and centrifuged at 1000 × g for 15 min. The supernatant (1 ml) was mixed with 4 ml of 2% Na_2CO_3, 0.1 N NaOH, 2% sodium potassium tartrate, 1% $CuSO_4$, and 10% Folin-Ciocalteu reagent (0.5 ml) and were incubated at room temperature (23 ±2°C) for 30 min in the dark. The absorbance of the solution was measured at 660 nm in a UV-Vis spectrophotometer (U-2000, Hitachi, Tokyo, Japan). The protein content of the plantains were calculated (using the standard curve of BSA) in terms of dry weight (D.W.) of plantains and expressed as mg/g.

2.2.3.4 Analysis of ash content

Ash contents of plantains were determined according to AOAC method 942.05 (1984). 4 g of sample was weighed in a silica dish and ignited on flame until fuming ceased. The dish was then transferred to a muffle furnace at 550°C and kept for 3 h followed by cooling in a desiccator. The sample was then weighed to evaluate the ash content.

2.2.4 Physicochemical assays of γ-irradiated green plantain

2.2.4.1 Texture profile analysis (TPA)

To analyze the changes in texture profiles of plantains with storage, TPA of whole plantains were conducted using a TA.XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). A two-bite test, as proposed by Mahajan and Goswami (2004), was conducted where plantains were compressed using the cylindrical (P/5) probe of diameter 6 mm to 20% of its original height with a test speed of 0.5 mm/s. From the TPA graph, parameters such as hardness, cohesiveness, chewiness, adhesiveness, springiness, gumminess and resilience of whole plantains were measured.

2.2.4.2 Determination of color profile

CIE color values (L*, a*, and b* values) of plantains were determined by CM-5 spectrophotometer (M/s Konica Minolta Inc., Osaka, Japan) at 10° inclination from light source. The color co-ordinates of these plantains were calibrated against a standard white plate. Chroma (C*) values and hue (h) angles were calculated using standard equations.

2.2.4.3 Pulp to Peel Ratio

Pulp to peel ratio is a consistent index of ripening of banana (Tourkey et al., 2014) and has been determined in this study in terms of ratio of weight of the pulp to the peel of the plantains.

2.2.4.4 Total Acidity (TA)

5 g of finely diced plantains (with peel) were homogenized using 25 ml double distilled water for 20 min. The homogenized sample (stock solution) was filtered and the filtrate obtained was used for assays of TA, pH and phytochemical characterizations. TA was determined by titrating the stock solution with 0.1N NaOH according to the method reported by Ranganna (2000) and was expressed in terms of % malic acid.

2.2.4.5 pH

pH of plantain stock solution was determined using pH meter (Cyberscan PC 510 m, M/s Eutech instruments Pte Ltd., Singapore) which has been standardized using standard pH buffer (4.01).

2.2.4.6 Reducing sugar

Reducing sugars in plantains were estimated in accordance with the methods elaborated by Pal and Bhattacharjee (2018), with few modifications. 5 g sample was homogenized with distilled water and centrifuged at 1000 x g for 15 min. The supernatant was acid hydrolyzed with HCl (6.34 N) in a water bath followed by neutralization with 1 N NaOH solution. A total of 0.8 ml of prepared sample was diluted with 3.2 ml water and 1 ml of freshly prepared DNSA reagent was added to the diluted sample. The mixture was then allowed to boil for 10 min, followed by immediate cooling in an ice-bath. The absorbance of the reaction mixture was measured using a UV–Vis spectrophotometer (U-2000; M/s Hitachi Corp., Kyoto, Japan) at 540 nm.

2.2.4.7 Leakage of ions from the γ-irradiated green plantains

The ability of membrane of a botanical to limit leakage of ions decreases with its senescence and is expressed in terms of percent membrane stability index (MSI) (Thakur et al., 2018). In this study, we have estimated the MSI in γ-irradiated plantains w.r.t. storage time using an electrical PC 510 conductivity meter (M/s Eutech Instruments, Malaysia) in accordance with the method reported by Chakrabarty et al. (2009).

2.2.4.8 Pectin content

Pectin content in cell walls of fruits is generally associated with fruit softening (Robinson and Sauco, 2010). Since plantains soften with senescence, pectin contents of plantains have been determined in terms of calcium pectate (Sadasivam and Manickam, 2005). Acid hydrolysis of plantains (50 g) was carried out with 400 ml of 0.05N HCl for 2 h at 80-90°C followed by filtration of the sample. Two aliquots of 200 ml each were drawn and water was added at a ratio of 250 ml per aliquot. The acid hydrolyzed sample was neutralized with 1 N NaOH solution. 10 ml excess 1N NaOH was added next and allowed to stand overnight. 50 ml of 1N acetic acid was added followed by the addition of 25 ml of 1N calcium chloride after 5 min and allowed to stand for 1h. This was boiled for 1 min and filtered through a previously weighed filter paper. The residue on the filter paper was washed with boiling water until chloride-free. The filter paper containing calcium pectate was dried and weighed.

2.2.5 Phytochemical assays of γ-irradiated green plantains

2.2.5.1 Estimation of DPPH radical scavenging activity

The antioxidant activities of the stock solutions of plantains were determined in terms of DPPH radical scavenging activities by spectrophotometric method (Aiyegoro and Okoh, 2010) and were expressed as IC_{50} values (mg/mL of plantains).

2.2.5.2 Determination of ferric reducing antioxidant power (FRAP) assay

The reducing abilities of the above-mentioned stock solutions of green plantains were determined by FRAP assay in accordance with the method reported by Chatterjee et al. (2013) and expressed as mM $FeSO_4$/g (D.W.) of green plantains.

2.2.5.3 Determination of total phenolic content (TPC)

TPC of the plantains was determined spectrophotometrically by Folin-Ciocalteu colorimetric method as described by Spanos and Wrolstad (1990). A standard curve of gallic acid was used to calculate the TPC in the samples and was expressed as mg gallic acid equivalent (GAE)/100 g D.W. of plantains.

2.2.6 Determination of ascorbate peroxidase (APX) and polyphenol oxidase (PPO) activities of γ-irradiated green plantains

2.2.6.1 APX activity

APX is one of the most important antioxidant enzymes in plants, which defends against peroxides and ROS during senescence. Therefore in this study, APX activities of the plantains have been estimated in γ-irradiated plantains during their storage period, in accordance with the method described by Ghosh et al. (2017). The enzyme activity was expressed in enzyme unit/mg protein.

2.2.6.2 PPO activity

PPO is responsible for browning reaction and discoloration in plantains. The enzymatic browning reaction that occurs in plantains during storage is due to the oxidation of phenolic compounds to brown pigments and can be determined by PPO activity (Olubunmi, 2013). The enzyme was extracted and assayed according to the method described by Galeazzi et al. (1981). 25 g of blended pulp was homogenized with 50 ml of 0.2 M sodium phosphate buffer solution of pH 7, containing 1% insoluble polyvinyl pyrrolidone (PVP) and 0.5% Triton X-100. The homogenate was centrifuged at 15000 x g for 15 min at 40°C. The enzyme activity was assayed in the supernatant by measuring the rate of increase in absorbance at 420 nm by spectrophotometer using catechol solution (0.1 M) as substrate. The enzyme activity was calculated from slope of the initial straight section of reaction curve and expressed as U /min/m.

2.3 Statistical analysis

All experiments were conducted in triplicate using three independent sets of plantains. Results were represented as mean ± SD of three independent experiments. Duncan's multiple range test was conducted to determine significant differences among means of different parameters of shelf life of plantains. A value of $p \leq 0.05$ was considered significant to establish differences in all tests. All statistical tests were performed by STATISTICA 8.0 software (Statsoft, Oklahoma, USA).

3. RESULTS AND DISCUSSION

3.1 Sensory evaluation of γ-irradiated green plantains

The sensory scores of the panelists for the quality attributes (color, odor, texture, shrinkage and overall appearance,) of γ-irradiated (0.02, 0.04, 0.06, 0.08, 0.1, 0.6, 1 kGy) and control sets of green plantains have been presented by radar plots in Figure 1. It has been observed that at day 0, all the sets have similar quality attributes. However a development of brown color in 0.6 and 1 kGy irradiated samples from day 2 onwards, suggested enzymatic browning in the same (discussed later). It was found that throughout the storage period (19 days) the scores of the panelists were highest for 0.04 kGy irradiated plantains, compared to others sets of plantain. Therefore 0.04 kGy has been considered to be the best dose of γ-irradiation, since the irradiated plantain had a lead of 5 days shelf life compared to control set (14 days)

3.2 Proximate compositions of γ-irradiated green plantains

3.2.1 Moisture content

The moisture content was found to decrease significantly ($p< 0.05$) in all sets of plantains (Figure 2) owing to the onset of ripening in the plantains. Similar trends in moisture loss in γ-irradiated and control sets of mushrooms have been reported by Thomas and Diehl (1988). However, the loss of moisture (31.25%) was found maximum in control set of plantains on day 14, compared to that in plantains γ-irradiated at 0.04 kGy (13.7%) on the same day. The above finding suggested delayed onset of ripening in the γ-irradiated plantain compared to the control sets.

3.2.2 Fat content

Fat contents in γ-irradiated and control sets of plantains were in the range of 0.49-0.52% (Figure 3). During storage, fat content did not change significantly ($p< 0.05$) in the all sets of plantains. This result is in agreement with Odenigbo et al. (2013), who had reported lipid content of plantains in the range of 0.41-0.7%.

3.2.3 Protein content

Protein content in plantain samples (both irradiated and control sets) decreased significantly ($p<0.05$) during storage. The increased concentrations of free radicals and ROS have reportedly been associated with membrane leakage and protein degradation in plants with senescence

(Sharma et al., 2012). Plantain γ-irradiated at 0.04 kGy had maximum protein content throughout the study. Therefore, it can be concluded that γ-irradiated plantains (0.04 kGy) were able to retain higher protein content than other sets of plantains during the storage period (Figure 4).

3.2.4 Ash content

Ash content in γ-irradiated and control sets of plantains did not change significantly ($p<0.05$) during the shelf-life study. Results indicated that mineral content in plantains was not affected by γ-irradiation. (Figure 5)

3.3 Physicochemical properties of γ-irradiated plantains

3.3.1 Texture profile of plantains

Hardness, adhesiveness and cohesiveness of plantains for both γ-irradiated and control sets were found to decrease; whereas, springiness and gumminess of the same sets were found to increase throughout the storage period. Among all the sets, plantains γ-irradiated at 0.04 kGy showed lower springiness, higher hardness, adhesiveness and cohesiveness than other sets during storage (Representative Figure 6a and 6b).This finding indicates retention of characteristic texture of the γ-irradiated plantains at the aforesaid dose, which corroborated well with the sensory attributes of the same (discussed earlier).

3.3.2 Color profile of plantains

Color analysis of the plantain samples are represented by L*, a*, b*, chroma (C*) and hue angles (h) (Table 1). a* and b* values obtained for the stored plantains suggested green color (indicated from negative a* value) of the same on day 0. However, with progression of storage, green color of plantains turned yellow, as has also been indicated by their higher b* values (28-38). Additionally, the increasing a* values (2.2 to 16.6, indicates browning) of the γ-irradiated as well as control sets of plantains have increased significantly ($p<0.05$) during the storage period, which suggested occurrence of browning in the above samples. Plantains irradiated at 0.04 kGy remained green (negative a*) up to day 14 and therefore suggested delayed onset of ripening in the above samples (Representative Figure 7a and 7b).

3.3.3 Peel to pulp ratio

The pulp to peel ratio of γ-irradiated and control sets of plantains were found to increase significantly (p<0.05) during storage (Figure 8). This finding was in agreement with Tourky et al. (2012), who have also reported increase in pulp to peel ratios during ripening of banana.

3.3.4 TA

TA of plantains in term of % malic acid was found to increase significantly (p<0.05) with storage time (Figure 9). This result was in agreement with Ayodele et al. (1983) who had reported similar trend in chemically treated plantains.

3.3.5 pH

pH values of γ-irradiated and control sets of plantains were in the range of 4.38-5.95 (Figure 10). No significant (p<0.05) change was observed in control and 1 kGy set of γ-irradiated plantains during the storage study. However, significant (p<0.05) decrease in pH was observed on the last day of shelf-life in plantains γ-irradiated in dose range of 0.02-0.6 kGy. This finding is in agreement with Giami and Alu (1993) who had also reported decreasing pH value (5.9 to 4.92) of the plantain during storage.

3.3.6 Reducing sugar content

Reducing sugar contents in plantains both γ-irradiated and control sets of samples increased significantly (p<0.05) with storage days, suggesting ripening of the above samples (Chamanee and Suntornwat, 1994). Control set had significantly (p<0.05) higher content of reducing sugar than γ-irradiated plantains (excluding 0.6 and 1 kGy) throughout their storage period (Figure 11), which indicated enhanced ripening in late senescence. This increase in the reducing sugar contents in the plantains is possibly due to hydrolysis of starch during ripening (Chamanee and Suntornwat, 1994).

3.3.7 Leakage of ions

Membrane stability was found to increase significantly (p<0.05) during initial days of storage (up to day 7) in γ-irradiated and control sets of plantains. However, from day 10, stability decreased significantly (p<0.05) in all the sets (Figure 12). These observations indicate that with

progression of storage, intracellular ions necessary for survival, leaked, owing to weakening of the cell membrane. Among all treated plantain, the highest MSI was observed in plantains γ-irradiated at 0.04 kGy. These findings attested 0.04 kGy dose to be the best dose of γ-irradiation in maintaining optimum membrane integrity during the storage period. Deterioration of proteins in botanicals (discussed earlier) can be attributed to increased membrane permeability and loss of ions (Fukuchi-Mizutani et al., 2000), which corroborated well with the present findings of percent MSI values.

3.3.8 Pectin content

Pectin content (in terms of calcium pectate) had decreased significantly ($p<0.05$) in both γ-irradiated and control plantain sets (Figure 13). Similar trend in reduction in pectin content (in terms of calcium pectate) in bananas have been reported by Kawabata and Sawayama (1974b). Plantains γ-irradiated at 0.04 kGy had retained appreciable pectin content during storage study. Wade et al. (1992) documented that pectin levels in mature bananas appeared to be lower than immature ones. However, the justification is not known, it needs further research.

3.4 Phytochemical properties of γ-irradiated green plantains

3.4.1 DPPH radical scavenging activity

The antioxidant activities in both γ-irradiated and control sets significantly ($p<0.05$) increased during initial days of storage, and decreased significantly ($p<0.05$) after day 10. Plantains γ-irradiated at 0.04 kGy were observed to have appreciable antioxidant activities, possibly owing to the highest melatonin (antioxidant) content in the same (elaborately discussed in section 3.1 of Chapter 1). 0.6 kGy irradiated plantains on day 10 of storage were observed to have highest antioxidant activities among all sets (Figure 14).

It is therefore opined that higher contents of L-tryptophan, serotonin and melatonin in 0.6 kGy on day 10 γ-irradiated plantains contributed to highest DPPH radical scavenging activity in the same indicating highest antioxidant activity (elaborated in section 3.1 of Chapter 1).

3.4.2 FRAP assay

The antioxidant activities of all plantain samples (both γ-irradiated and control sets) obtained by FRAP assay, was found to increase significantly (p<0.05) during the initial days of storage. However, antioxidant activity decreased significantly (p<0.05) after day six. 0.04 kGy γ-irradiated plantains were observed to have appreciable antioxidant activities (probably due to highest melatonin content); whereas, 0.6 kGy γ-irradiated plantains had significantly (p<0.05) high antioxidant activity during the study (Figure). The obtained result was due to enhanced contents of L-tryptophan, serotonin and melatonin in 0.6 kGy plantains (as discussed under DPPH radical scavenging activity). The findings of FRAP assay of plantains corroborated well with those obtained by DPPH radical scavenging assay (Figure 15).

3.3.3 TPC

TPC of the γ-irradiated and control sets showed decreased significant (p<0.05) during the shelf-life period (Figure 16). This phenomenon of accumulation and reduction of phenolic compounds in γ-irradiated post-harvest produce could be attributed to the alterations in its cellular structure and metabolism during initial days of study and oxidative degradation of the same during later days as has been reported by Wi et al. (2007).

3.5 APX and PPO activities of γ-irradiated green plantains

3.5.1 APX activity

APX activities significantly (p< 0.05) increased in γ-irradiated and control sets up to day 10 (Figure 17). However after day 10, APX activities decreased. Similar results have also been reported by Pal and Bhattacharjee (2016) who reported that APX activity in γ-irradiated marigold flowers increased during storage up to day 8, after which the activity started to decline. This phenomenon is commonly observed in plants wherein during initial days of storage, the ascorbate peroxidase enzyme activity increases to avert oxidation in cells; however, during late senescence, the enzyme activity decreases owing to increased ROS concentration, resulting from enhanced H_2O_2 in plant cells (Wi et al., 2007).

3.5.2 PPO Activity

The PPO activities of γ-irradiated and control sets of plantains were found to increase significantly (p<0.05) during storage (Representative Figure 18). Giami and Alu (1993) found similar trend in increase of PPO activities of plantains during ripening. From the above result, it can be inferred that PPO activity increases with ripening and browning of plantains (observed from the color and texture profiles of the same). Browning of the plantains that occurred was mainly due to oxidation of phenolic substances to melanin catalyzed by PPO (Thomas and Nair, 1970).

4. CONCLUSION

The present study revealed that 0.04 kGy γ-irradiated plantains had appreciable sensory scores, with minimum ripening and retained a fresh green color for a period of 19 days, with a lead of 5 days compared to the control set. Therefore it could be reasonably concluded that the effective dose of γ-irradiation for plantains was 0.04 kGy. The extension in shelf life of γ-irradiated plantains would improve export and allow farmers to fetch appropriate market value of their produce.

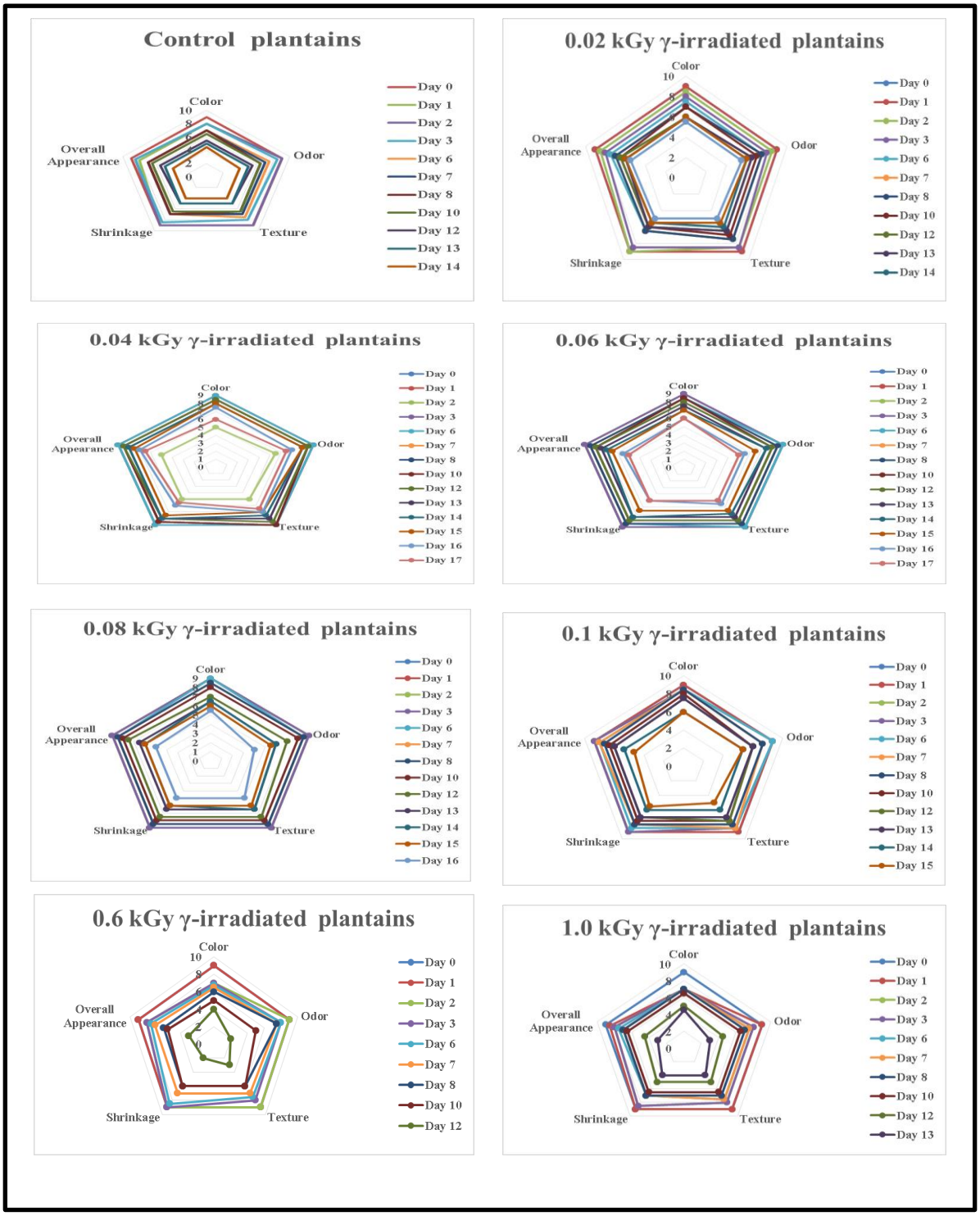

Figure 1: Radar plots for sensory evaluation of control and γ-irradiated plantains

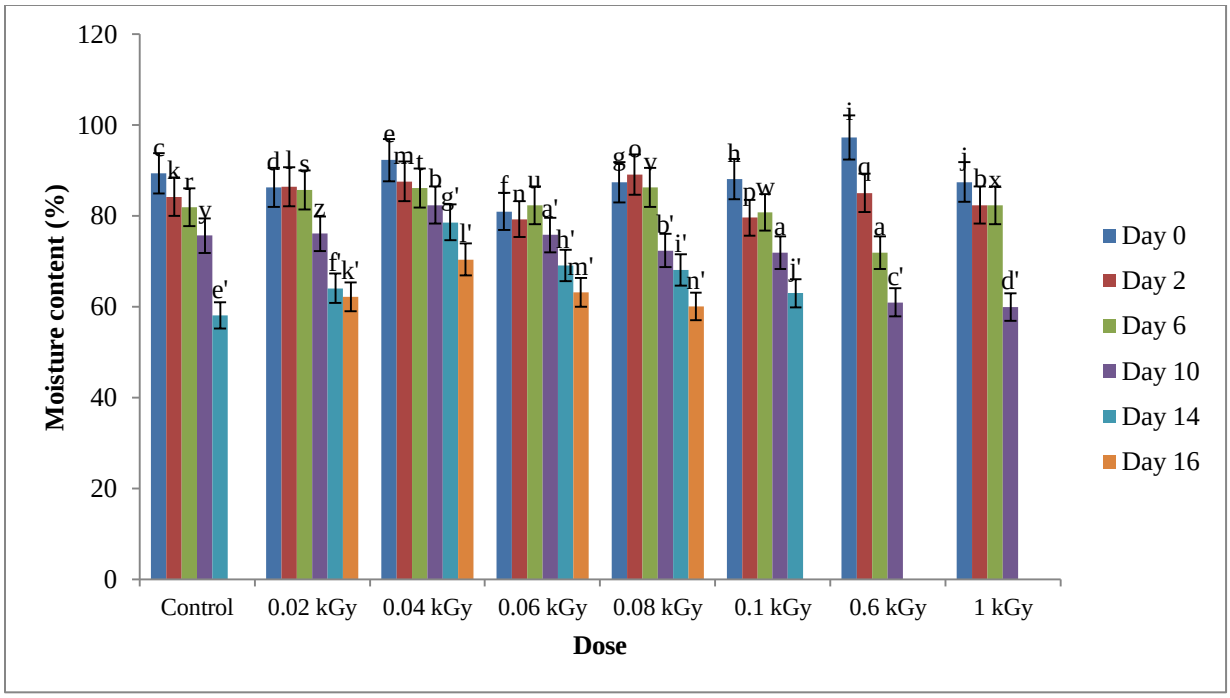

Figure 2: Changes in moisture contents of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p < 0.05$

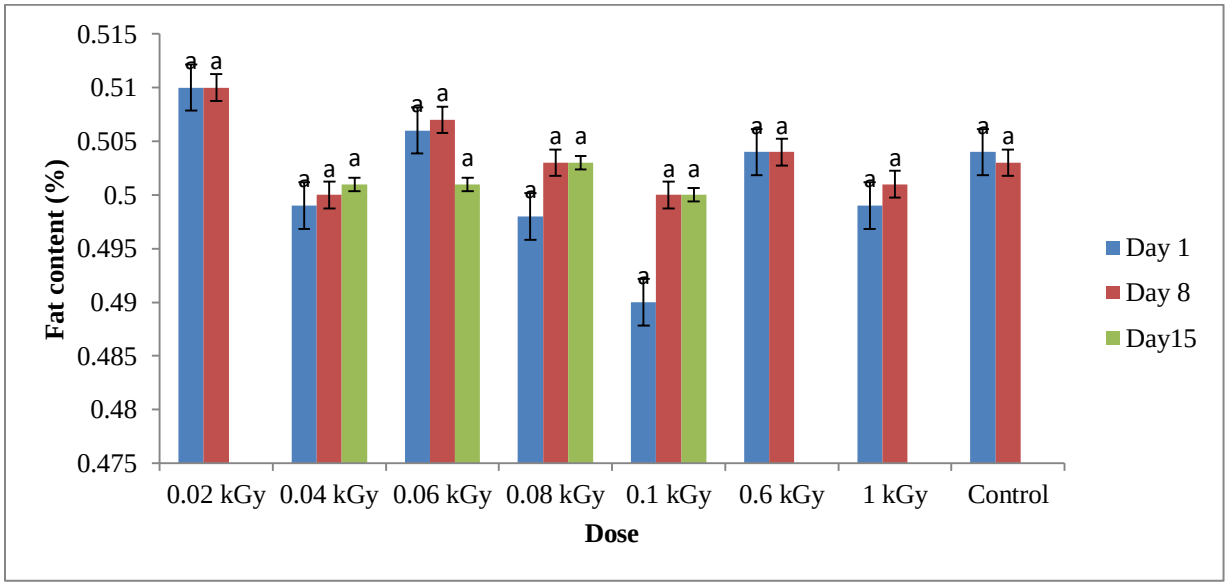

Figure 3: Changes in fat contents of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p < 0.05$

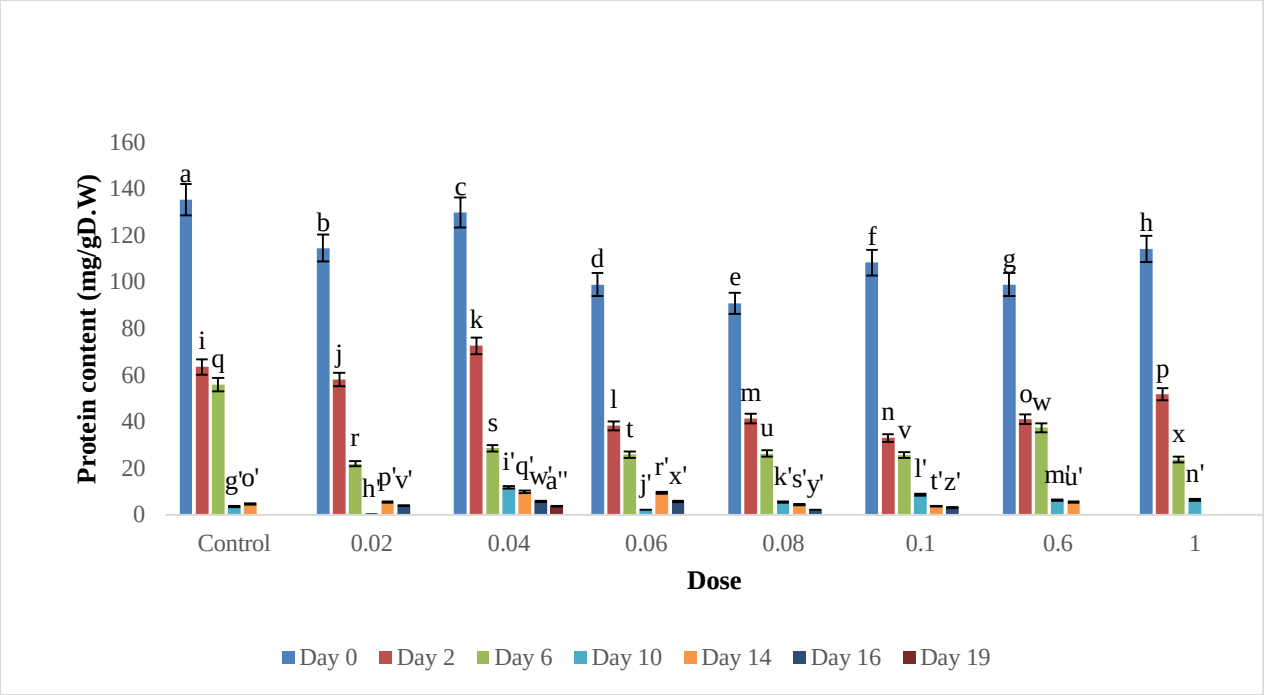

Figure 4: Changes in protein contents of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

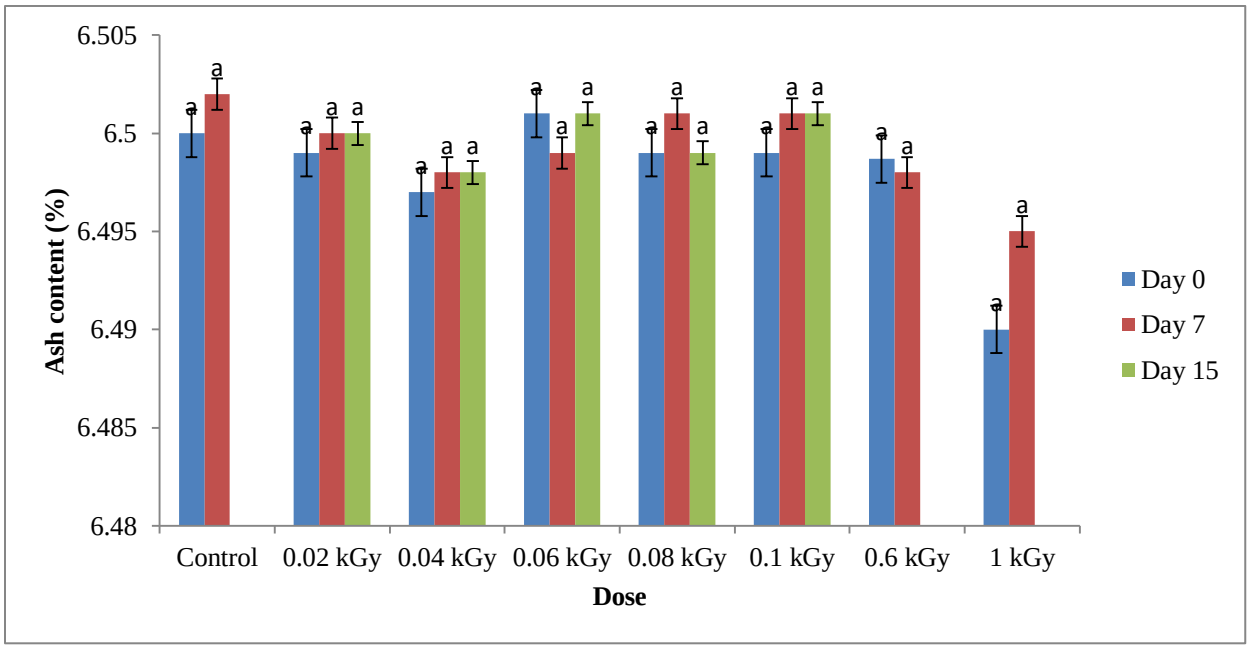

Figure 5: Changes in ash contents of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

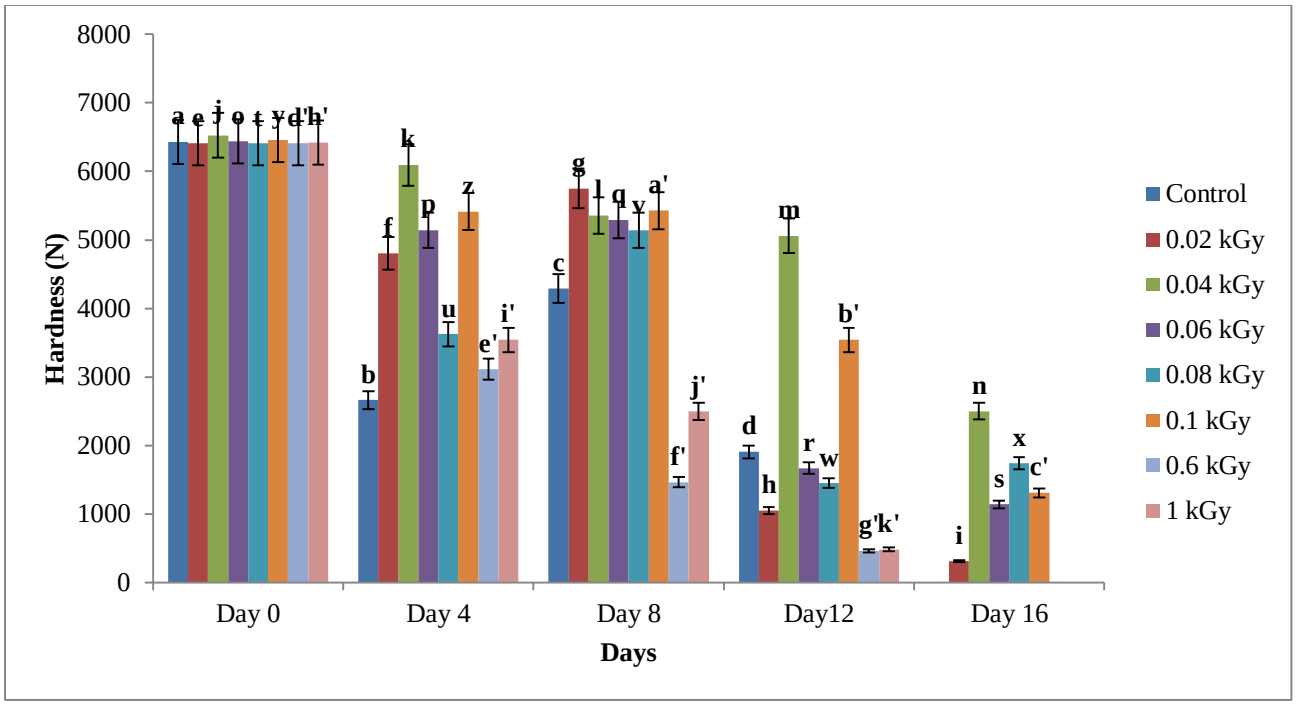

Figure 6a: Changes in Hardness values of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

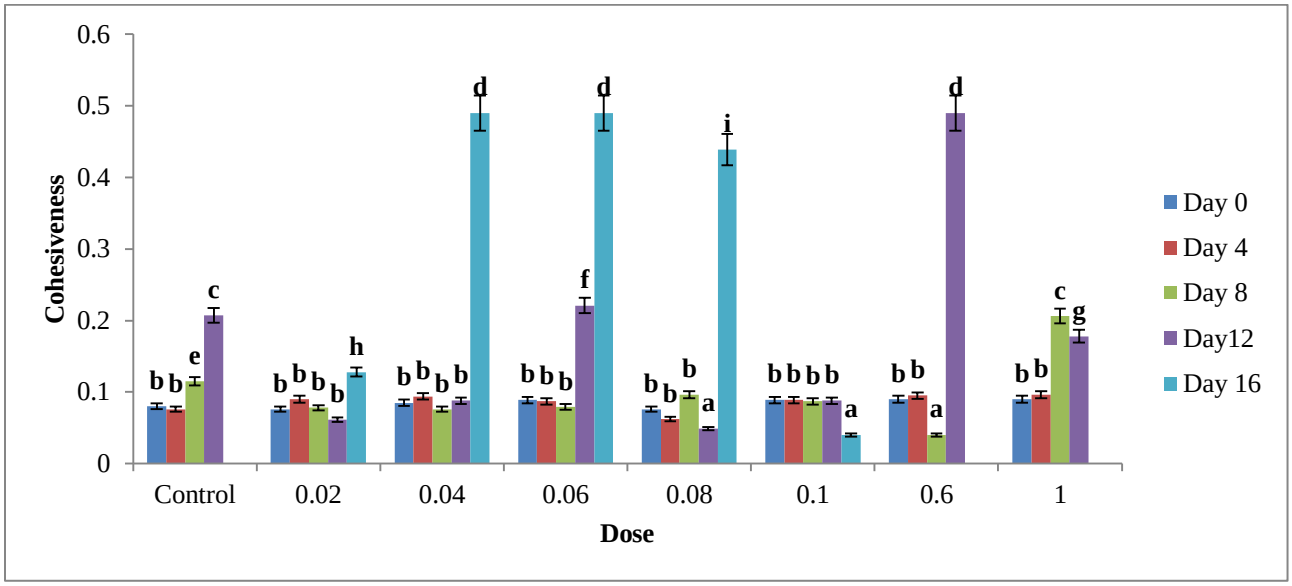

Figure 6a: Changes in Cohesiveness values of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

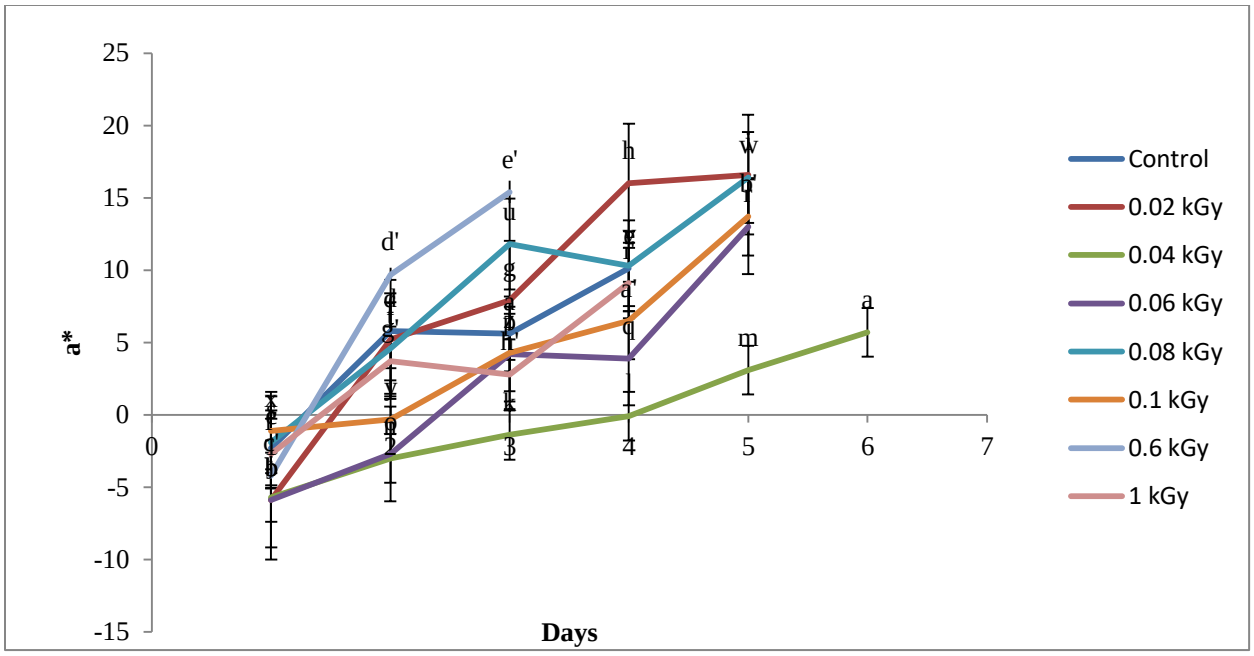

Figure 7a: Changes in a* values of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

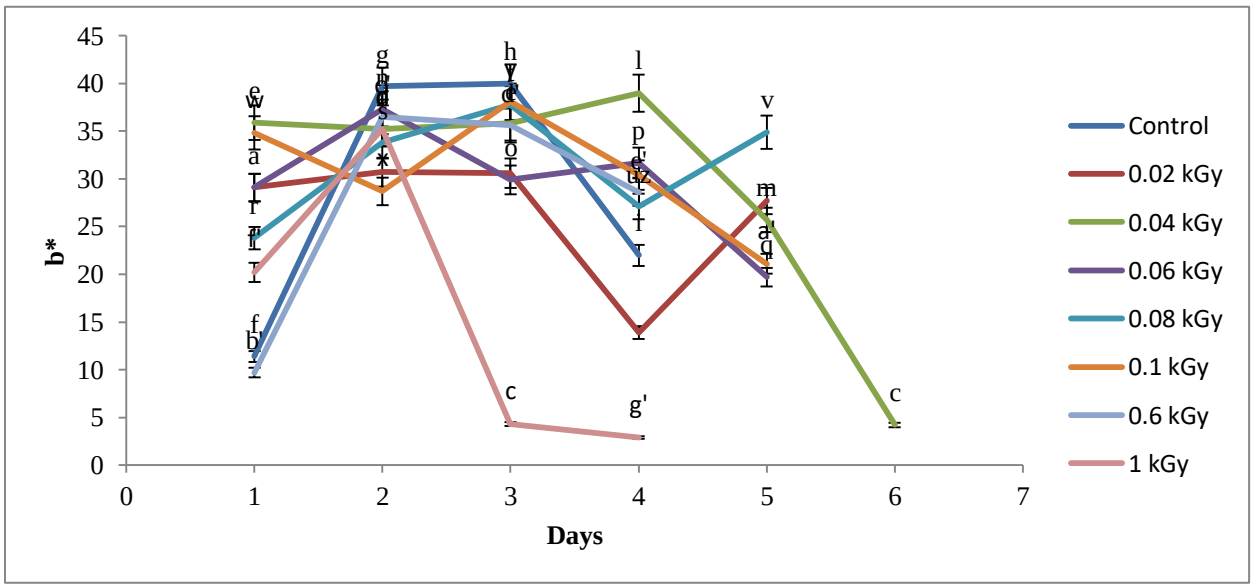

Figure 7b: Changes in b* value of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

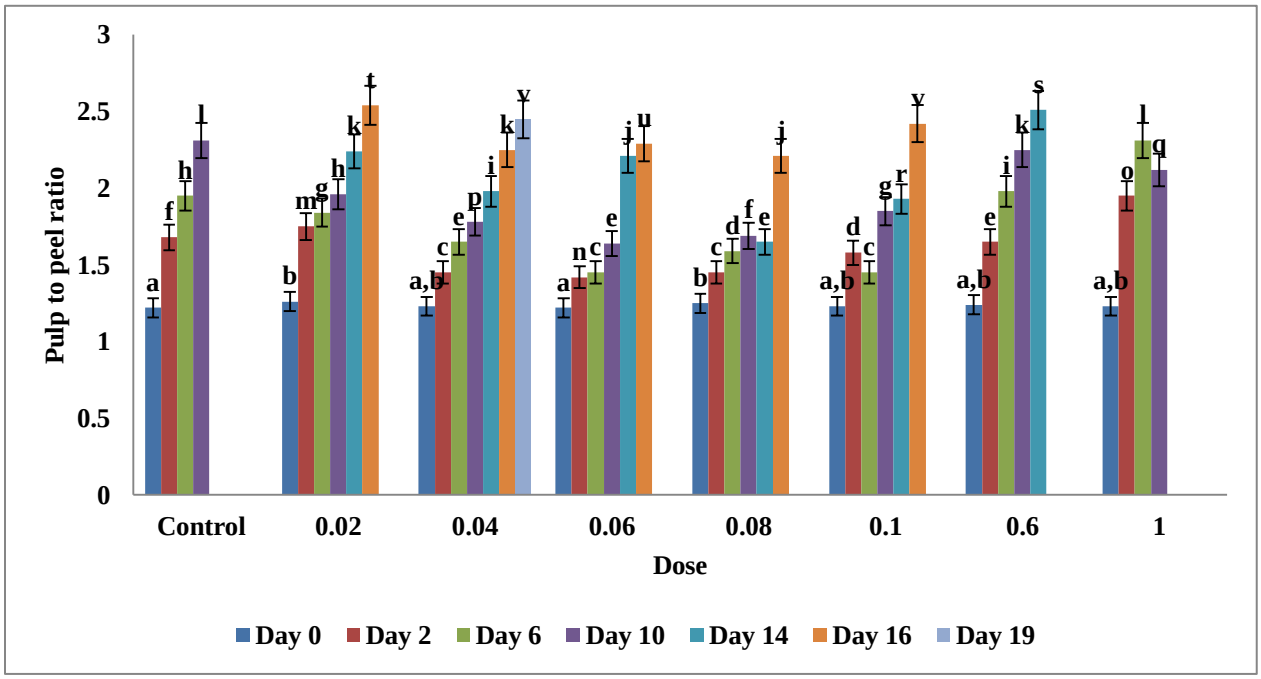

Figure 8: Changes in pulp to peel ratio of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p< 0.05$

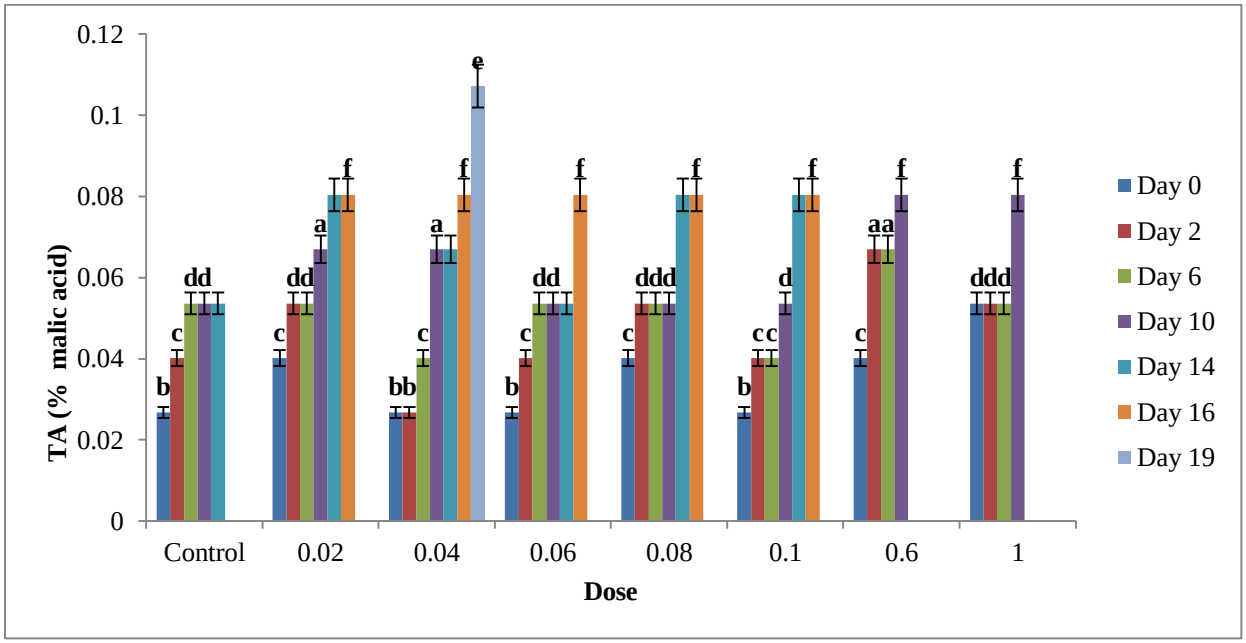

Figure 9: Changes in TA as % malic acid of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p< 0.05$

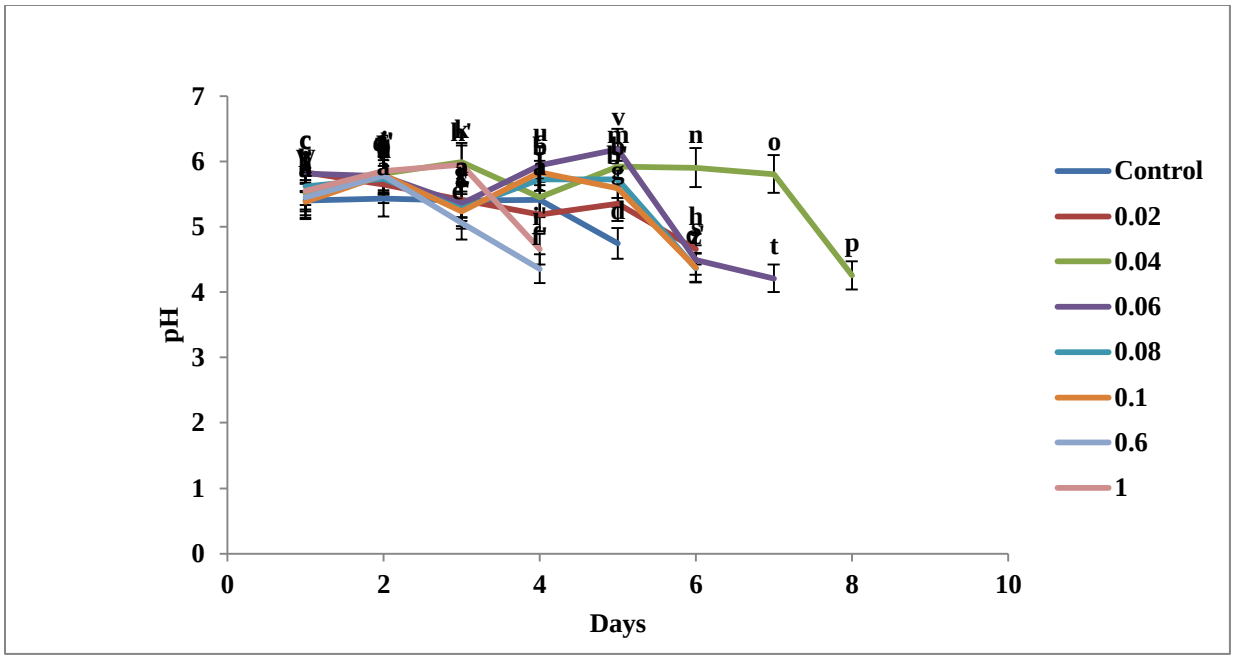

Figure 10: Changes in pH of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p < 0.05$

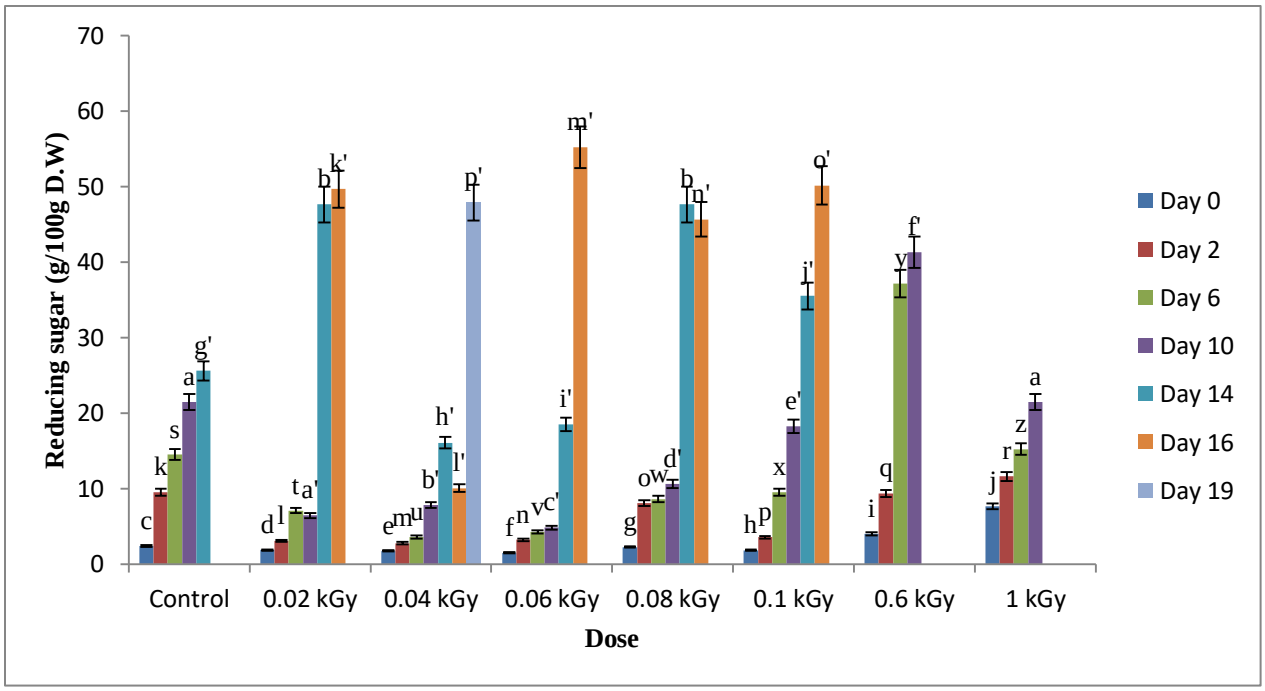

Figure 11: Changes in reducing sugar content of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p < 0.05$

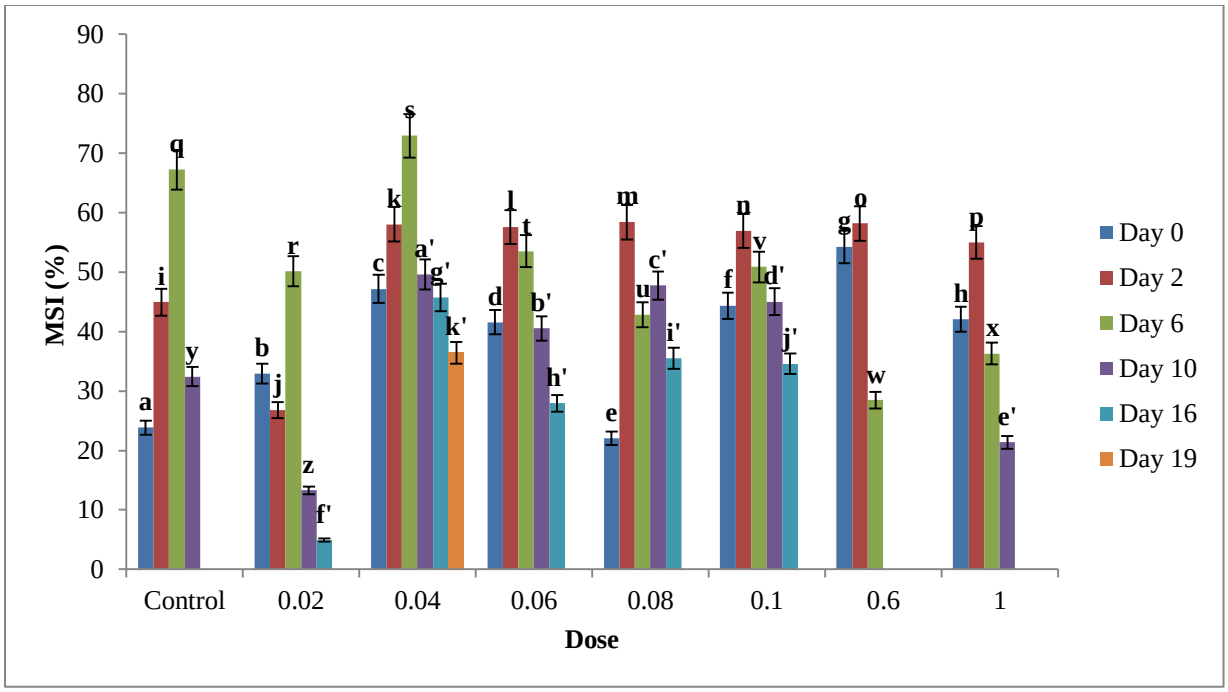

Figure 12: Changes in % MSI of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

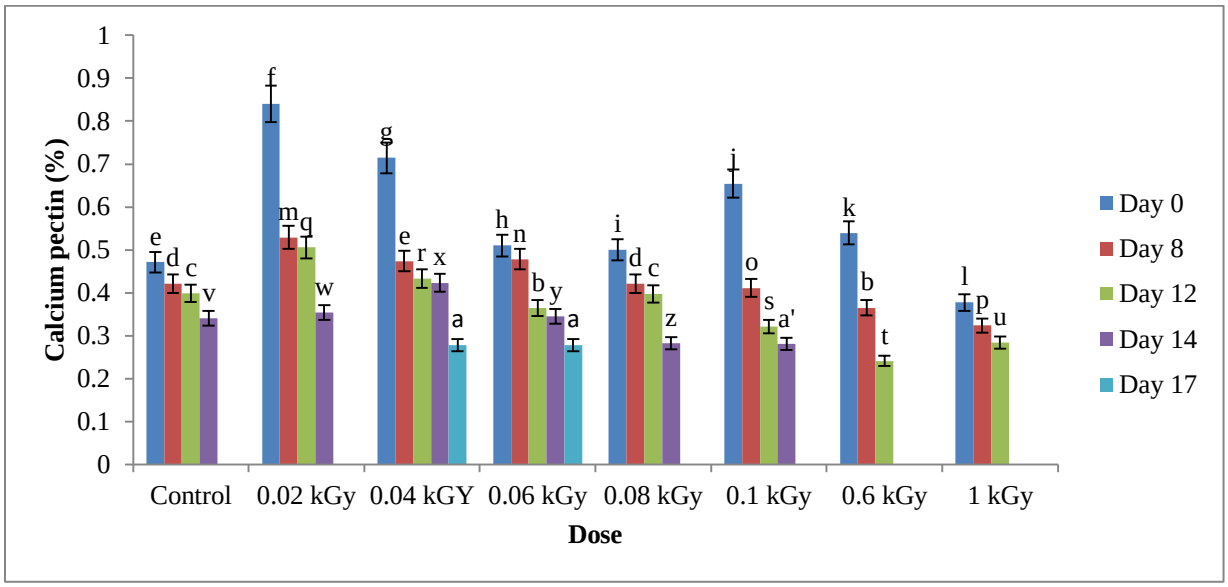

Figure 13: Changes in pectin content as % calcium pectate of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

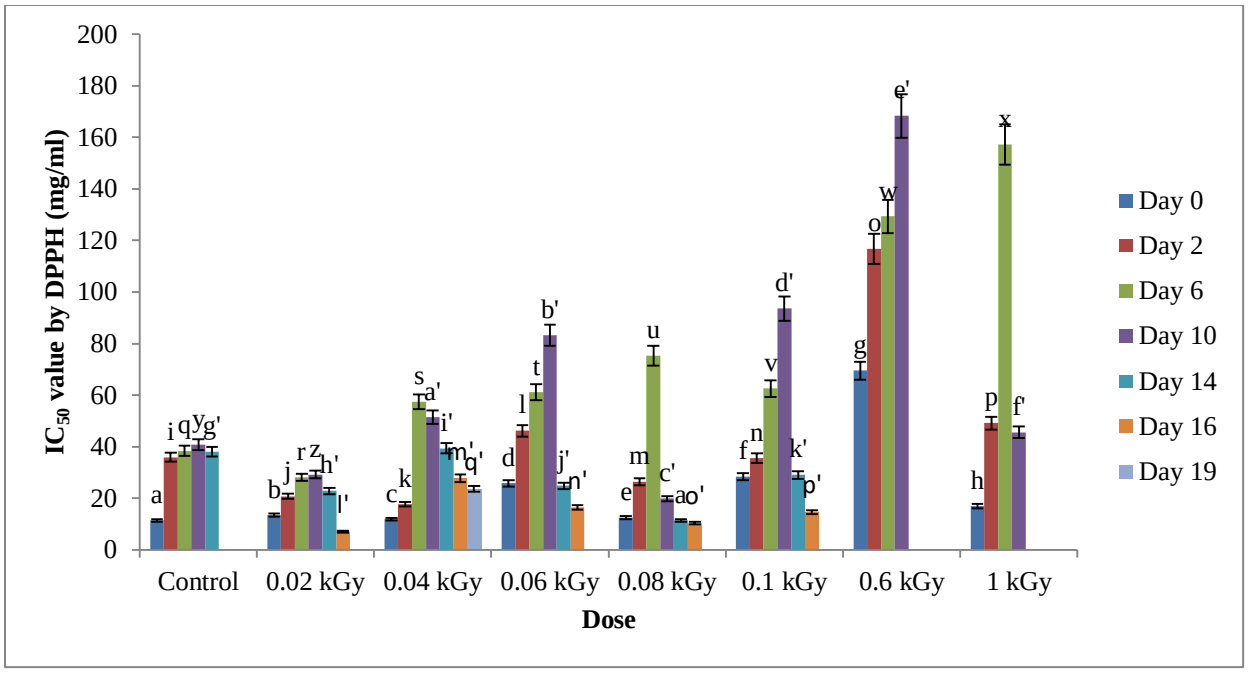

Figure 14: Changes in IC_{50} value by DPPH of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p< 0.05$

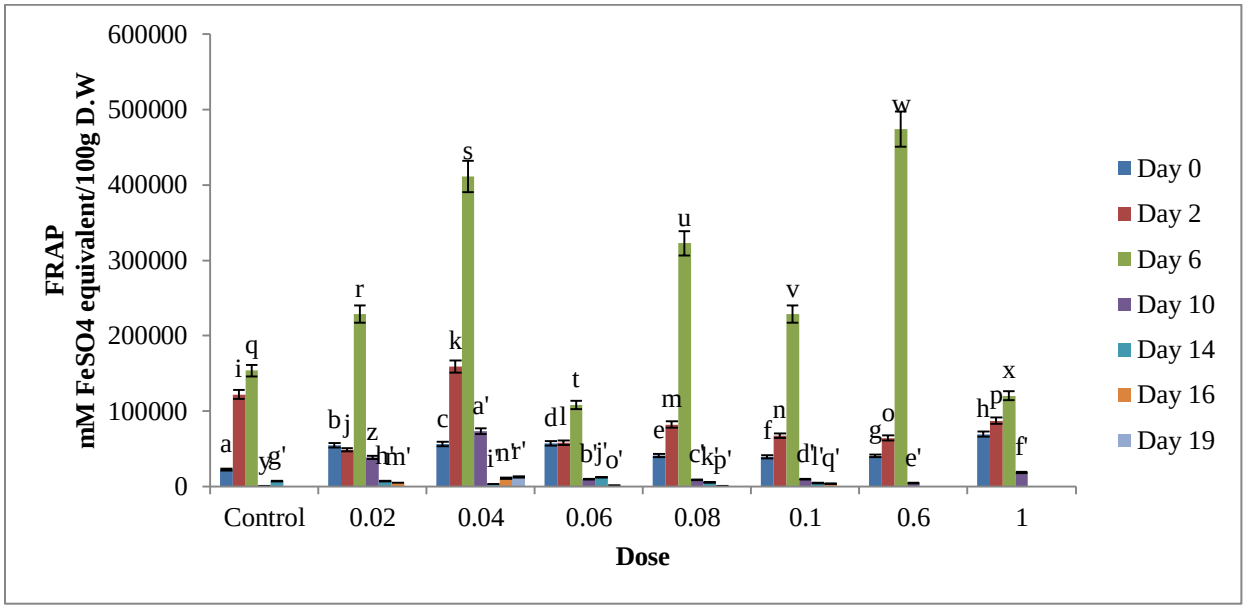

Figure 15: Changes in FRAP values of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p< 0.05$

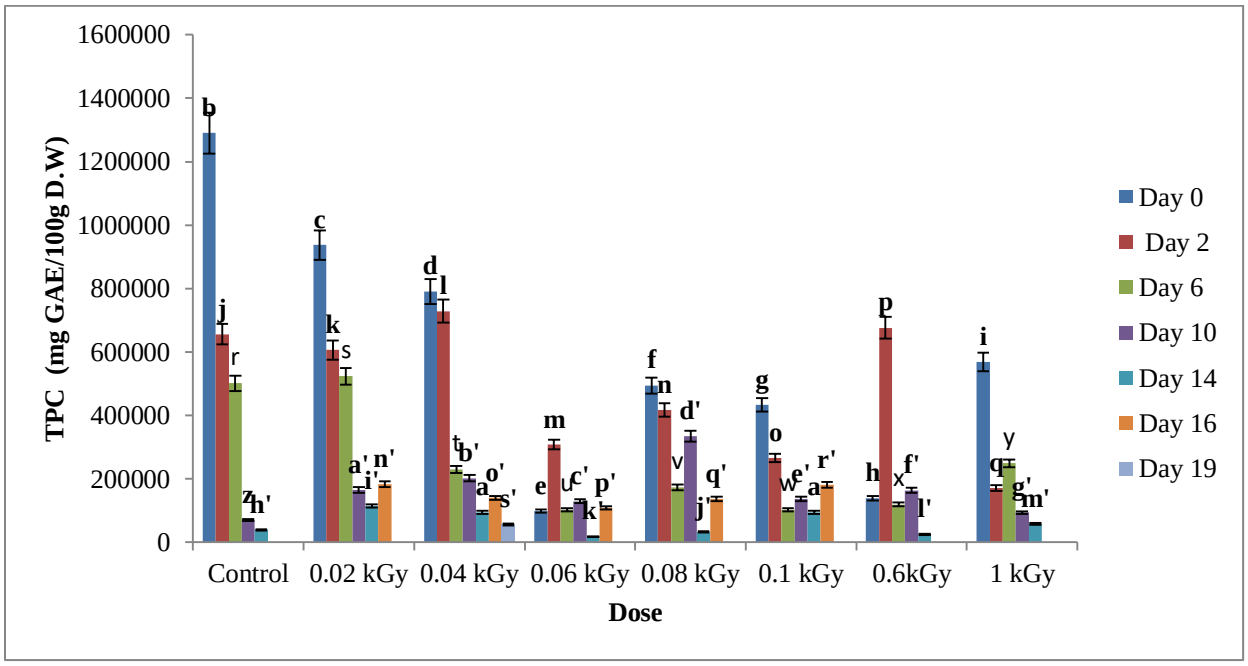

Figure 16: Changes in TPC of control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

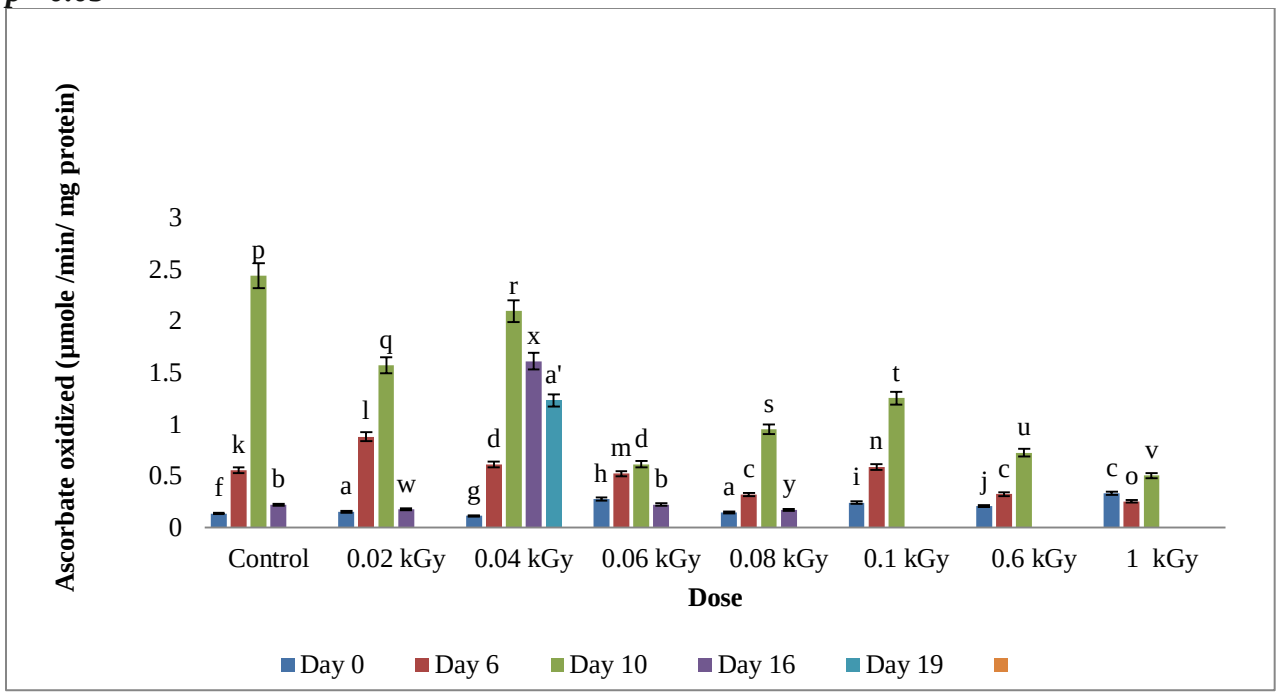

Figure 17: Ascorbate oxidized in control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at *p*< 0.05

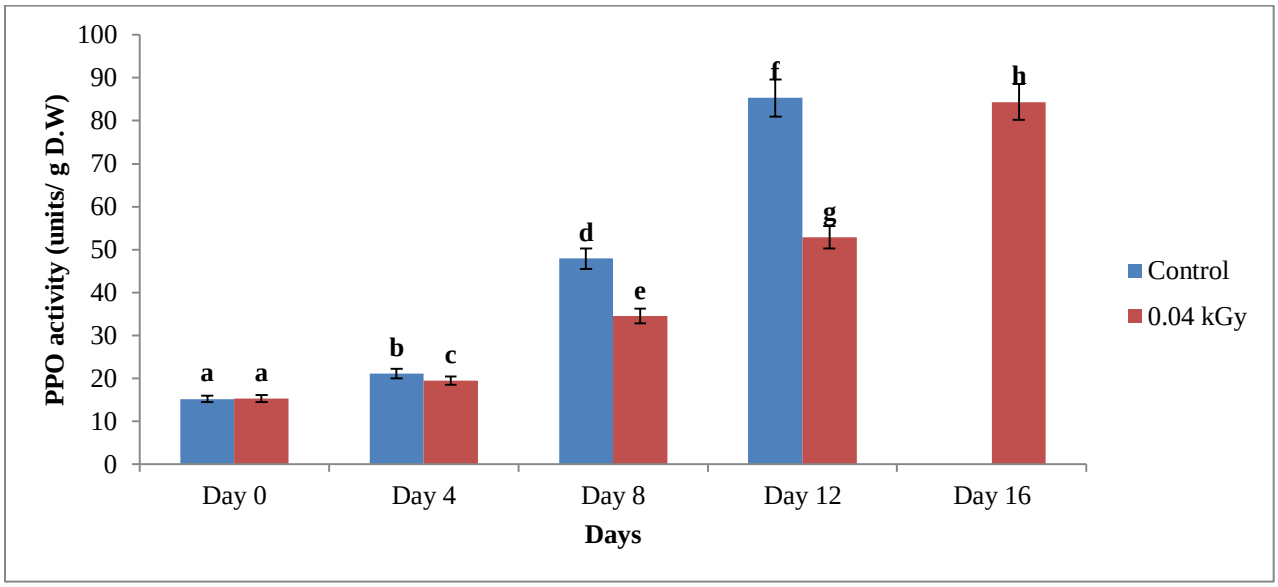

Figure 18: PPO activity in control and γ-irradiated plantains during storage. Each value represents the mean ± SD of three independent experimental data of three batches of plantains. Dissimilar alphabets indicate that the mean values belong to different subsets at $p < 0.05$

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SUMMARY AND FUTURE PROSPECTS

This study focused on the application of γ-irradiation in extending the shelf-life of γ-irradiated *desi* variety of Indian plantains and also in enhancing the biomolecules present in green plantains namely L-tryptophan, serotonin and melatonin. Green plantains were γ-irradiated in a selective dose range of (0.02-1 kGy) for optimization of doses to enhance the aforesaid biomolecules and for extension of shelf-life of the irradiated plantains in terms of sensory attributes, proximate, physicochemical and phytochemical properties during storage. After γ-irradiation, extraction of the biomolecules from the green plantains were conducted by SPE method and simultaneously quantified by HPLC. From the above mentioned studies, it was observed that 0.6 kGy γ-irradiated green plantains had significantly higher contents of L-tryptophan, serotonin and melatonin among plantains irradiated in the dose range of 0.02-1 kGy and their non-irradiated counterparts. In order to validate the HPLC analysis, mass transfer of the biomolecules in HPLC packed bed in terms of dimensionless numbers namely Re, Sc and Pe numbers were conducted. The data generated from the above studies were used to validate the HPLC method used for simultaneous quantification of low amounts of L-tryptophan, serotonin and melatonin. The findings indicate that the method of quantification by HPLC was unambiguous and reliable in this study and this method can be used for quantification of L-tryptophan, serotonin and melatonin, extracted from other fruits and vegetables.

Among plantains γ-irradiated in the range of 0.02-1kGy, it was observed that 0.04 kGy γ-irradiated plantains had highest shelf-life of 19 days with a lead of 5 days, vis-à-vis its non-irradiated counterpart. Due to high shelf-life, these γ-irradiated plantains would improve export and allow farmers to fetch appropriate market value of their produce. Among all doses of irradiation investigated, 0.6 kGy γ-irradiated plantains owing to their highest antioxidant activities (due to enhanced contents of antioxidants namely serotonin and melatonin) could be utilized as a safe source of these food antioxidants.