

# Effects of Gamma Irradiation on Starch Granule Structure and Physicochemical Properties of Unripe *Musa acuminata* and *Musa cavendish*

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## Abstract

The present study was aimed to isolation of starch and modified by gamma-irradiation at 5 kGy dose. The objective of the study was to investigate the SEM, FTIR, amylose content and physico-chemical and functional, flow properties. The  $L^*$ ,  $a^*$  (4.56-2.54), and  $b^*$  (11.81-9.49) values were also investigated for both starches. The results revealed that the flow properties of both starches were found to be poor. The moisture (7.9-10.23 %), ash, and fat content were decreased while fibre (0.71-0.94 %) increased with the doses. Whereas the WAC (7.57-11.48 g/g), OAC (8.13-11.66 g/g) increased while emulsion activity (11.16-5.37 %) and stability (65.21-42.24 %) were decreased with the dose. The swelling power was decreased while the solubility index was increased with the dose. Freeze-thaw stability, syneresis, and transmittance were decreased with the dose. The SEM and FTIR study indicated that tightly packed, the formation of patterns and cracks, fractures, breaches, cavities, and holes were seen in modified starch.

**Keywords:** Starch, Freeze-thaw, Gamma-irradiation, Amylose, Fat

## 1. INTRODUCTION

Banana (*Musa paradisiaca*), a member of the *Musa* family, is an edible fruit widely cultivated in tropical and subtropical regions. It is a hybrid polyploid derived from wild plantains and is highly perishable and seasonal. The genus *Musa* includes multiple species and varieties, such as the red banana (*Musa acuminata*), known for its reddish skin and rich antioxidant content. This variety is cultivated in regions like Australia, Brazil, and Central America, and in India, primarily in Kerala and Tamil Nadu. Red bananas are rich in phenolic compounds, tannins, anthocyanins, minerals, and natural antioxidants, which contribute to their color, aroma, and health benefits [1]. Another variety, the green banana (*Musa Cavendish*), is high in starch and valued for its role as a functional carbohydrate. These are largely cultivated in developing nations across Asia, Africa, and Latin America. India ranks as the second-largest producer of bananas after mangoes, contributing approximately 21.87% of the nation's total fruit production and 10.49% of the regional output. According to the FAO, India leads global banana production with 24.87 million tonnes, followed by China (10.55 million tonnes) and Brazil (6.9 million tonnes). Nutritionally, bananas are rich in essential minerals such as calcium, iron, magnesium, sodium, potassium, and phosphorus, as well as phytochemicals like sterols and unsaturated fatty acids. Their high potassium and low sodium content make them ideal for blood pressure regulation. Furthermore, the presence of vitamins, resistant starch (RS), polysaccharides, and sugars makes bananas beneficial in reducing the risk of cardiovascular diseases, strokes, and certain cancers [2]. Starch, a semi-crystalline polymer composed of amylose and amylopectin, is the primary carbohydrate reserve in higher plants and is widely consumed. Amylose consists of linear  $\alpha$ -

1,4-linked glucose chains, whereas amylopectin has both  $\alpha$ -1,4- and  $\alpha$ -1,6-linked chains, creating a branched structure. The modification of starch resulting from processing methods, environmental conditions, or operational parameters can significantly influence its physicochemical, morphological, functional, and pasting properties. Modifications are typically classified into three types: physical, chemical, and enzymatic. Physical modifications, such as irradiation, are considered safer than chemical modifications, which alter molecular structures and may restrict their use in food applications [3,4]. Irradiation is a non-thermal, physical method used for food preservation and safety enhancement. This technique involves exposing food to controlled doses of ionizing radiation, similar to other physical processes like freezing or pasteurization. Gamma irradiation, using sources such as Cobalt-60 ( $^{60}\text{Co}$ ) and Cesium-137 ( $^{137}\text{Cs}$ ), is particularly effective for microbial decontamination and pest control. It can also induce structural modifications in starch through polymer degradation, grafting, or crosslinking mechanisms. According to the Codex Alimentarius Commission (CAC), over 40 countries currently use irradiation for more than 60 types of food products available in global markets, highlighting its increasing relevance in food safety and preservation [5].

## **2. METHODOLOGY**

### **2.1. Samples Collection**

The unripe bananas (*Musa acuminata* and *Musa Cavendish*) procured from the local market outlet in Delhi (I.N.A sabzi mandi) and damaged pieces of banana were removed manually by visual inspection and an experiment was conducted in the laboratory. All chemicals utilized in this study were of analytical grade.

### **2.2. Starch isolation**

Unripe bananas (*Musa acuminata* and *Musa Cavendish*) were washed, peeled, and diced into small pieces before being blended into a paste. The filter was maintained for 2 hours, centrifuged at 8400 rpm for 10 minutes, water was removed, and starch in wet form was collected and dried in a hot air oven at 60°C. Grind the dried starch by using a mortar pestle and store it in a zipper pouch for subsequent analysis.

### **2.3. Gamma irradiation treatment**

About 300 g of dried banana starches were packaged and subjected to 5 kGy doses of gamma irradiation by using cobalt-60 as a source of radiation it was performed at the Shri Ram Institute for Industrial Research, New Delhi, India.

## **2.4. PHYSICO-CHEMICAL ANALYSIS**

### **2.4.1. Proximate composition**

The analysis of moisture and ash content was conducted according to the standard procedure [6].

### **2.4.2. Fat, Protein and Fibre**

The quantification of fat, protein, and fibre content was conducted using the automatic fat analyzer Kelplus [7].

### **2.4.3. Carbohydrate**

The technique outlined in [25] was utilized to quantify the carbohydrate.

### **2.4.4. Amylose**

The amylose content of starch was assessed using the method outlined in reference [8].

### **2.5. Color Characteristics**

The sample's color values, expressed as  $L^*$ ,  $a^*$ , and  $b^*$ , were quantified using a color meter (Shenzhen 3nh Technology Co., Ltd, China) under illuminant D65 with 100 observers.

## **2.6. FUNCTIONAL PROPERTIES**

### **2.6.1. Absorption of water and oil**

The water absorption capacity (WAC) of the samples (1 g) was assessed according to the method [11], while the oil absorption capacity (OAC) was evaluated using the procedure specified in [12,13] with centrifuge equipment.

### **2.6.2. Solubility Index and Swelling Power**

The water solubility index (WSI) and swelling power (SP) were assessed at temperatures of 60, 70, 80, and 90 °C, following the sample modification [14].

### **2.6.3. Light Transmittance**

1 g sample was measured by using a slightly modified method, after which the suspension was stored under refrigerated conditions at 4°C for 5 days. The transmittance was assessed every 24 h at 640 nm against distilled water as a blank by UV-spectrophotometer [15].

### **2.6.4. Syneresis**

Syneresis was assessed using the modified technique described in reference [16].

### **2.6.5. Emulsion Activity and Stability**

The samples were conducted according to the techniques outlined in [8] by using 10 ml of distilled water, and 10 ml of soybean oil.

### **2.6.6. Foam Capacity and Stability**

The technique outlined in [17] was utilized to assess the foam capacity (FC) and foam stability (FS) of both native and modified starch.

### **2.6.7. Least gelation concentration**

The least gelation concentration (LGC) was ascertained utilizing the modified approach suggested [18].

### **2.6.8. Freeze thaw stability**

The freeze-thaw stability of starch was assessed using the method with few modifications [19].

## **2.7. Scanning electron microscopy (SEM)**

The morphological characteristics of the starch samples were analyzed using scanning electron microscopy (SEM) at the Indian Institute of Technology (IIT), New Delhi, India. A 1% starch suspension in ethanol was affixed onto aluminum stubs using double-sided adhesive tape. The samples were then coated with a thin layer of gold-palladium to enhance conductivity. Imaging was performed using a ZEISS EVO 50 SEM operated at an accelerating voltage of 20 kV.

## **2.8. FTIR (Fourier Transform Infrared (FTIR) spectroscopy)**

The Fourier transform infrared (FTIR) spectra of starches were obtained using an FTIR spectrophotometer from the Japan Spectroscopic Company. The starch sample was combined with KBr and formed into pellets before measurement. KBr was utilized as a blank during calibration from 500 to 4000 cm<sup>-1</sup> spectrum.

## **2.9. X ray diffraction**

The XRD patterns of the starch samples were obtained using an Empyrean PAN analytical diffractometer, equipped with a pixel detector utilizing CuK- $\alpha$  radiation, operating at 45 kV and 40 mA. The samples were analyzed from  $2\theta = 2^\circ$  to  $90^\circ$  with a step interval of  $0.05^\circ$  and a scan rate of  $20^\circ$  per minute.

## **2.10. STATISTICAL ANALYSIS**

The data for native and modified banana starch are shown as means of three replicates. The samples will be analyzed in triplicate, and data collected for specified quality indicators will be analyzed for mean and standard deviation. The data were assessed for statistical significance at  $p < 0.05$  using one-way analysis of variance (ANOVA) followed by Duncan's test, employing IBM SPSS Statistics 20 software.

## **3. RESULT AND DISCUSSION**

### **3.1. Effect on moisture content**

The effect of treatments R0, R1, T0, and T1 on the physicochemical properties of banana starch is presented in Table 1. Moisture content ranged from 7.9% to 10.23%, with the highest value observed in R0 (10.23%) and the lowest in T1 (7.9%). A progressive reduction in moisture content was noted with increasing doses of gamma irradiation. R1 and T0 showed intermediate values of 9.50% and 8.24%, respectively. This decline in moisture aligns with previous findings and suggests that gamma irradiation did not negatively impact moisture retention. Native starch samples (R0 and T0) exhibited higher moisture content compared to their irradiated counterparts (R1 and T1), indicating a dose-dependent effect. Variations may be attributed to

varietal characteristics and regional environmental conditions. Since moisture content plays a key role in food preservation where values above 13% can encourage microbial growth and spoilage at lower moisture content is desirable for extended shelf life.

### 3.2. Effect on Fat

The fat content of the samples was found to be 0.61% (R0), 0.49% (R1), 0.47% (T0), and 0.32% (T1), with R0 showing the highest and T1 the lowest values. A consistent decreasing trend in fat content was observed with increasing gamma irradiation dosage, aligning with previous studies [12,13]. This reduction may be attributed to structural alterations in the starch matrix, particularly the disruption of fatty acid double bonds caused by irradiation. Elevated fat levels in starch can lead to rancidity, off-flavors, oxidative degradation, and microbial susceptibility. The observed reduction in fat content post-irradiation suggests improved storage potential and oxidative stability of the treated starch samples.

### 3.3. Effect on Protein

The protein content of R0, R1, T0, and T1 ranged from 0.38% to 0.72%, with the lowest in R0 and highest in T1. A progressive increase in protein levels was observed with rising  $\gamma$ -irradiation doses, consistent with findings in peach starch [20]. This enhancement is attributed to free radical generation during irradiation, which may alter protein structure and conformation. Additionally, low radiation doses may improve the digestibility and albumin/globulin fractions without significantly affecting the electrophoretic profile.

**Table 1. The physico-chemical properties of native and irradiated banana starch**

Parameters	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
<b>Moisture (%)</b>	10.23±0.05 <sup>d</sup>	9.50±0.03 <sup>c</sup>	8.24±0.05 <sup>b</sup>	7.9±0.05 <sup>a</sup>
<b>Ash (%)</b>	0.31±0.06 <sup>b</sup>	0.14±0.03 <sup>a</sup>	0.61±0.04 <sup>c</sup>	0.4±0.05 <sup>b</sup>
<b>Fat (%)</b>	0.61±0.04 <sup>b</sup>	0.57±0.02 <sup>b</sup>	0.45±0.04 <sup>a</sup>	0.47±0.02 <sup>a</sup>
<b>Protein (%)</b>	0.38±0.03 <sup>a</sup>	0.66±0.04 <sup>c</sup>	0.57±0.05 <sup>b</sup>	0.72±0.05 <sup>c</sup>
<b>Fibre (%)</b>	0.94±0.05 <sup>b</sup>	0.89±0.07 <sup>b</sup>	0.72±0.06 <sup>a</sup>	0.71±0.04 <sup>a</sup>
<b>Amylose (%)</b>	31.52±0.06 <sup>b</sup>	28.81±0.04 <sup>a</sup>	37.15±0.04 <sup>d</sup>	35.67±0.05 <sup>c</sup>
<b>pH</b>	6.07±0.02 <sup>a,b</sup>	6.24±0.58 <sup>b,c</sup>	6.72±0.04 <sup>c</sup>	5.53±0.04 <sup>a</sup>
<b>Carbohydrate (%)</b>	89.39±0.11 <sup>c</sup>	89.85±0.56 <sup>c</sup>	87.51±0.21 <sup>a</sup>	88.21±0.06 <sup>b</sup>
<b>Energy (kcal)</b>	363.96±0.30 <sup>c</sup>	366.55±2.71 <sup>c</sup>	357.13±0.38 <sup>a</sup>	360.69±0.44 <sup>b</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>=Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

Value expressed are means ± SD (n = 3).

Means in the rows with different superscripts are significantly different ( $p \leq 0.05$ ).

### 3.4. Effects on Ash

Ash content ranged from 0.14% (R1) to 0.61% (T0), with a general decline observed with increasing  $\gamma$ -irradiation doses. Ash represents the mineral residue left after combustion. The results align with previous findings in  $\gamma$ -irradiated cassava and wheat starch, where ash content ranged from 0.12% to 0.14% [13, 20, 21]. The reduction may be associated with moisture loss and mineral degradation due to irradiation.

### 3.5. Effect on Fibre

The present analysis revealed that R<sub>0</sub> (0.94%) possessed the highest fibre content, whilst T<sub>1</sub> exhibited the lowest fibre level at 0.71%. The  $\gamma$ -irradiation may alter the crystalline regions of the fibres, disrupt the fibre matrices and crystallization zones, and degrade the covalent bonds, potentially resulting in the loss of fibre structure. Fibre is essential in the advancement of functional meals.

### 3.6. Effect on pH

The pH of the starches ranged from 5.53 to 6.72 across all samples. The maximum pH was recorded in T<sub>0</sub>

(6.72), whilst the minimum was in T<sub>1</sub> (5.33). The pH decreased with increasing radiation dose, likely due to the cleavage of glycosidic bonds by free radicals produced during irradiation of the starch.

### 3.7. Effect on Amylose

The amylose content for R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> was recorded as 31.52%, 28.81%, 37.15%, and 35.67%, respectively. Starches can be classified into four types based on amylose content: waxy (0–2%), semi-waxy (3–15%), normal (20–35%), and high-amylose (>40%) [22]. As shown in Figure 4.3, amylose content varied with different irradiation doses of banana starch. Similar trends were observed in talipot palm, potato, and bean starch, where amylose content decreased with increased irradiation [23,16]. A comparable pattern was also reported for two potato starch types white and red where amylose content decreased from 32.60 to 24.20 g/100 g and from 32.00 to 22.60 g/100 g, respectively [24]. Variations in amylose levels may depend on starch variety, growth conditions, and maturity. Gamma irradiation affects amylose content by promoting its interaction with amylopectin, which increases junction zones and gel hardness. However, this can also lead to partial gelatinization, causing granule collapse and a softer gel. Additionally, lipid–amylose complex formation can reduce retrogradation. The increased amylose content might also be due to hydrolysis of amylopectin, exposing linear chains. Despite this, gamma rays can degrade amylose and reduce its iodine-binding ability, leading to lower apparent values. Thus, irradiated starch is suitable in bakery applications for enhancing softness and stability post-baking.

### 3.8. Color Index

The L\* values for samples R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> ranged 72.7, 75.62, 71.75, and 73.72, respectively, as displayed in Table 2. The current investigation indicated that the lightness (L\*) values of R<sub>0</sub> and T<sub>0</sub> ranged from 72.7 to 71.75, which were generally lower than those reported for Honduras banana starch [26]. In the current study, the L\* values for R<sub>1</sub> and T<sub>1</sub> varied from 75.62 to 73.72, exhibiting a little increase, while a\* and b\* values for R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> demonstrated a decreasing trend, with values of 2.60, 2.54, 4.59, and 2.95, and 10.66, 10.43, 11.81, and 9.49, respectively. Comparable outcomes for the 'L\*' value were noted for the Indian Horse Chestnut referenced by [27]. The values of a\* for R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> were positive, attributed to a faint red hue and relatively elevated values observed in R<sub>0</sub> and T<sub>0</sub>. Comparable results were noted, with a positive value of a\* in buckwheat and potato starch reported by [9].

A positive b\* value signifies the existence of a yellow component in starches. According to numerous studies, the b\* value indicates increased ash content, and the current results support this concept, demonstrating that the b\* value diminished with increasing dose as the ash content likewise fell following radiation. The caramelization reaction of monosaccharide produced from starch polysaccharides via gamma-irradiation is responsible for the effects of gamma-irradiation [8]. The polyphenolic substance, ascorbic acid, and carotene associated with the starch impart color to the starch, so affecting its quality. Certain color tints in the starch are transferred to the end product, resulting in diminished quality. Consequently, the color of the finished product is a significant criterion; thus, the present investigation shown that neither native nor modified starches are suitable for industrial applications.

**Table 2. Color quest of native and irradiated banana starch**

Parameters	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
L*	71.75±0.06 <sup>a</sup>	73.72±0.05 <sup>c</sup>	72.7±0.04 <sup>b</sup>	75.62±0.04 <sup>d</sup>
a*	4.59±0.04 <sup>c</sup>	2.95±0.02 <sup>b</sup>	2.60±0.05 <sup>a</sup>	2.54±0.05 <sup>a</sup>
b*	11.81±0.05 <sup>d</sup>	9.49±0.03 <sup>a</sup>	10.66±0.09 <sup>c</sup>	10.43±0.04 <sup>b</sup>
Whiteness index	96.82±0 <sup>b</sup>	96.87±0.005 <sup>d</sup>	96.7±0 <sup>a</sup>	96.85±0 <sup>c</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>= Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

Values expressed are means ± SD (n = 3).

Means in the rows with different superscripts are significantly different (p ≤ 0.05).

#### 4.1. Effect on Flow Properties of Banana Starches

The flow characteristics of banana starch are critical for assessing its applicability as direct compression excipients [28]. As shown in Table 3, the bulk density (BD) of samples R0, R1, T0, and T1 was found to be 0.48 g/cc, 0.50 g/cc, 0.48 g/cc, and 0.48 g/cc, respectively. Their tapped density (TD) values were 0.64 g/cc for R0 and R1, 0.65 g/cc for T0, and 0.66 g/cc for T1. The higher TD compared to BD indicates better packing upon tapping, which can be attributed to factors such as particle size, geometry, solid density, and surface properties [29]. A higher TD is advantageous for packaging, allowing more material to be stored in a fixed volume [30]. The findings suggest no significant changes in BD and TD due to gamma ( $\gamma$ ) irradiation, implying that the starch polymer structure was not adversely affected. However, the overall low density values indicate that the starch granules might be non-porous or that more packaging volume may be required, which is a crucial consideration for storage and transport. Carr's Index values for R0, R1, T0, and T1 were 24.7%, 20.38%, 26.13%, and 35.84%, respectively, while Hausner's Ratio values were 1.32, 1.25, 1.26, and 1.55. These results are depicted in Figure 4.6. Both Carr's Index and Hausner's Ratio are indirect indicators of flow behavior, where a Hausner's Ratio above 1.25 and a Carr's Index above 25% suggest poor flow properties [31]. The present study confirms cohesive behavior in both raw and treated banana starch at all irradiation levels, with T1 particularly showing the poorest flow. Although the angle of repose remained below 50° a threshold for poor flow so the Carr's Index exceeded 35% in T1, further suggesting suboptimal flow. The flowability of starches is also influenced by their moisture content and water absorption capacity, with higher values leading to increased cohesion and reduced flow [32,33].

**Table 3. The flow properties of native and irradiated banana starch**

Parameters	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
Bulk density (g/cc)	0.48±0.02 <sup>b</sup>	0.50±0.01 <sup>c</sup>	0.42±0.004 <sup>a</sup>	0.48±0.01 <sup>b</sup>
Tapped density (g/cc)	0.64±0.02 <sup>a</sup>	0.64±0.024 <sup>a</sup>	0.65±0.02 <sup>a</sup>	0.66±0.03 <sup>a</sup>
Carr's index (%)	25±4.07 <sup>a,b</sup>	21.87±0.51 <sup>a</sup>	35.38±2.29 <sup>b</sup>	27.27±2.90 <sup>c</sup>
Hausner ratio	1.32±0.07 <sup>a</sup>	1.25±0.005 <sup>a</sup>	1.26±0.17 <sup>a</sup>	1.55±0.07 <sup>b</sup>
Angle of repose (°)	63.47±0.01 <sup>a</sup>	63.47±0.04 <sup>a</sup>	63.47±0.07 <sup>a</sup>	63.43±0.01 <sup>a</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>= Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

Values expressed are means ± SD (n = 3).

Means in the rows with different superscripts are significantly different ( $p \leq 0.05$ ).

#### 4.2. Effect on Functional Properties of Banana Starch

##### 4.2.1. Water and Oil absorption capacity

The data variation in the water absorption capacity and ability to absorb oil of the individual starches. The WAC of R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> varied from 7.57 % to 11.48 % among all the types of individual starch as presented in Table 4. Water absorption capacity was observed highest in T<sub>1</sub> (11.48%) and R<sub>1</sub> (11.21%) and lowest in R<sub>0</sub> (7.57%) and T<sub>0</sub> (11.01%). WAC is an important parameter that has been implicated in viscosity and also maintains the consistency of the product as well as baking properties. The current study showed the capacity to absorb water was enhanced with the increased dose due to the breakdown of starch granules to simpler sugars with a greater affinity for water than starch, such as maltose, dextrins, and others [9]. Water absorption capacity was directly correlated with the hydrolysis time and acid concentration which may increase the molecular weight of the starch fraction with the H group which having the ability to entrap the water molecules may result in increasing the water absorption capacity. These higher values are associated with higher starch content whose matrix will demand more H<sub>2</sub>O during hydrolysis than sugar molecules [34]. The oil absorption capacity of different starches was noted as R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> are 8.13 %, 11.66 %, 10.67 %, and 11.44 % respectively. In the present study, R<sub>1</sub> and T<sub>1</sub> showed the highest value as compared to T<sub>0</sub> and R<sub>0</sub>. It is known that OAC is the physical entrapment of oil which means oil bound by matrices in a particular food. OAC is responsible for flavor retention and mouth feel, palatability improvement, and shelf-life extension [35]. The observed increased oil absorption capacity of irradiation starch may be connected to the ability for damaged and/or cross-linked starch to trap oil. Higher values found in water and oil absorption

could be ascribed to the starch granule composition that could hold the water and oil within therefore, present findings suggested that it may be useful in food applications such as the formation of bakery products like cake and cookies, etc.

**Table 4. The functional properties of native and irradiated banana starch.**

Parameters	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
Water absorption capacity (g/g)	7.57±0.06 <sup>a</sup>	11.21±0.03 <sup>c</sup>	11.01±0.10 <sup>b</sup>	11.48±0.07 <sup>d</sup>
Oil absorption capacity (g/g)	8.13±0.07 <sup>a</sup>	11.66±0.05 <sup>d</sup>	10.67±0.04 <sup>b</sup>	11.44±0.05 <sup>c</sup>
Emulsion activity (%)	8.81±0.06 <sup>c</sup>	5.37±0.05 <sup>a</sup>	11.16±0.05 <sup>d</sup>	7.60±0.07 <sup>b</sup>
Emulsion stability (%)	48.17±0.05 <sup>b</sup>	42.24±0.05 <sup>a</sup>	65.21±0.05 <sup>d</sup>	56.53±0.07 <sup>c</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>= Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

Values expressed are means ± SD (n = 3).

Means in the rows with different superscripts are significantly different (p ≤ 0.05).

#### 4.2.2. Effect on Emulsion activity and stability

The emulsion activity of banana starch samples R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> was recorded as 8.81%, 5.37%, 11.16%, and 7.60%, respectively, while the emulsion stability (ES) values were 48.17%, 42.24%, 65.21%, and 56.53%, respectively. Emulsion stability refers to the rate at which the oil and water phases separate during storage, whereas emulsion activity reflects the maximum oil a protein can emulsify. The present study revealed a decreasing trend in both emulsion activity and stability with increasing doses of  $\gamma$ -irradiation. These emulsion properties are closely associated with the amylose content of starch. In food systems composed of proteins, carbohydrates, fats/oils, and water, multiple interfaces exist, and stable emulsions are essential to maintain texture and structure. Enhanced emulsion properties help reduce oxidation, prolong shelf life, and improve texture by maintaining smoothness and structural integrity. However,  $\gamma$ -irradiation appeared to negatively impact these properties. According to [36,7], this reduction in emulsion stability might be attributed to the disintegration and aggregation of protein molecules under radiation exposure, thereby impairing the emulsifying potential of the starch.

#### 4.2.3. Effect on swelling power and solubility index

The swelling power for banana starch samples R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> ranged from 8.77–16.45 g, 7.81–15.07 g, 10.38–17.17 g, and 9.16–15.84 g, respectively, across temperatures from 50°C to 90°C, as presented in Table 5. Swelling power and solubility index increased with rising temperature for all samples. However, a reduction in swelling power was observed in the 5 kGy-irradiated samples (R<sub>1</sub> and T<sub>1</sub>), likely due to varietal effects. The highest swelling power was noted at 90°C for R<sub>0</sub> (16.45 g) and T<sub>0</sub> (17.17 g), while the lowest values were observed for R<sub>1</sub> (7.81 g) and T<sub>1</sub> (9.16 g) at 50°C. These findings are consistent with earlier studies [12,9]. The decline in swelling power in irradiated samples (R<sub>1</sub> and T<sub>1</sub>) suggests that  $\gamma$ -irradiation alters starch granule structure, potentially forming a dense starch gel matrix in which the liquid phase is confined, reducing swelling [27]. A higher swelling power is generally desirable as it indicates better starch digestibility and broader dietary applicability. The decrease in swelling is also linked to radiation-induced breakdown of starch, particularly amylopectin, which reduces the swelling index [37,35]. By limiting granule rupture, this reduction may help retain better texture upon cooking. Irradiation is reported to fragment amylopectin's outer chains, leading to lower molecular weight and reduced swelling power [38]. Maximum solubility for all samples was observed at 90°C, while the lowest was at 50°C, consistent with prior results [16,39]. Both temperature and irradiation dosage enhanced solubility. Improved solubility with increased amylose content is due to granule disruption, which facilitates hydrogen bonding between starch molecules and water as amylose leaches into solution. This trend has been similarly observed in sweet potato, cowpea, and potato starches [40,9,24], and also in native and irradiated kithul starch within the 60–90°C and 0.5–10 kGy dose range. Horse chestnut and broad bean starches also showed comparable increases in solubility [27,39]. The elevated solubility in irradiated starches is attributed to radiation breaking intermolecular hydrogen bonds, promoting water interaction. Furthermore, radiation-induced depolymerization yields mono- and disaccharides with higher water affinity, thereby enhancing

solubility [41].

**Table 5. Swelling power and Solubility index of native and irradiated banana starch.**

Parameters	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
<b>Swelling power (g/g)</b>				
50°C	8.77±0.07 <sup>b</sup>	7.81±0.04 <sup>a</sup>	10.38±0.03 <sup>d</sup>	9.16±0.04 <sup>c</sup>
60°C	11.25±0.04 <sup>c</sup>	9.16±0.04 <sup>a</sup>	12.61±0.06 <sup>d</sup>	10.35±0.03 <sup>b</sup>
70°C	13.38±0.05 <sup>c</sup>	11.52±0.04 <sup>b</sup>	14.37±0.05 <sup>d</sup>	11.16±0.04 <sup>a</sup>
80°C	15.35±0.04 <sup>c</sup>	14.21±0.04 <sup>b</sup>	16.54±0.04 <sup>d</sup>	13.25±0.04 <sup>a</sup>
90°C	16.45±0.04 <sup>c</sup>	15.07±0.03 <sup>a</sup>	17.17±0.05 <sup>d</sup>	15.84±0.05 <sup>b</sup>
<b>Solubility index (%)</b>				
50°C	1.48±0.54 <sup>a</sup>	3.24±0.05 <sup>c</sup>	2.60±0.07 <sup>b</sup>	4.25±0.03 <sup>d</sup>
60°C	3.66±0.09 <sup>a</sup>	5.41±0.04 <sup>b</sup>	5.71±0.22 <sup>c</sup>	7.23±0.05 <sup>d</sup>
70°C	4.67±0.05 <sup>a</sup>	7.50±0.07 <sup>c</sup>	6.41±0.06 <sup>b</sup>	9.47±0.03 <sup>d</sup>
80°C	7.65±0.02 <sup>a</sup>	8.61±0.06 <sup>b</sup>	9.35±0.03 <sup>c</sup>	11.38±0.04 <sup>d</sup>
90°C	9.71±0.04 <sup>a</sup>	10.71±0.06 <sup>c</sup>	10.38±0.05 <sup>b</sup>	12.36±0.05 <sup>d</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>= Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

Values expressed are means ± SD (n = 3).

Means in the rows with different superscripts are significantly different (p ≤ 0.05).

#### 4.2.4. Effect on Syneresis

Syneresis refers to the expulsion of water from a gel over time during storage. The syneresis values for R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> ranged from 1.71–42.58 %, 1.12–40.59 %, 2.88–46.21%, and 2.10–41.17 %, respectively, as presented in Table 6. The lowest syneresis was observed during the 1st cycle and the highest during the 5th cycle of frozen storage. R<sub>0</sub> and T<sub>0</sub> showed the maximum syneresis values compared to their irradiated counterparts (R<sub>1</sub> and T<sub>1</sub>), indicating that gamma irradiation reduced syneresis.

**Table 6. Syneresis of native and irradiated banana starch.**

Syneresis (%)	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
0 h	1.71±0.06 <sup>b</sup>	1.12±0.04 <sup>a</sup>	2.88±0.07 <sup>d</sup>	2.10±0.04 <sup>c</sup>
24 h	13.47±0.03 <sup>b</sup>	11.31±0.04 <sup>a</sup>	16.22±0.04 <sup>c</sup>	13.49±0.05 <sup>b</sup>
48 h	18.27±0.04 <sup>b</sup>	16.42±0.06 <sup>a</sup>	21.87±0.06 <sup>d</sup>	19.3±0.04 <sup>c</sup>
72 h	30.7±0.04 <sup>b</sup>	28.75±0.05 <sup>a</sup>	33.11±0.06 <sup>d</sup>	32.60±0.04 <sup>c</sup>
96 h	42.58±0.04 <sup>c</sup>	40.59±0.06 <sup>a</sup>	46.21±0.05 <sup>d</sup>	41.17±0.05 <sup>b</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>= Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

Values expressed are means ± SD (n = 3).

Means in the rows with different superscripts are significantly different (p ≤ 0.05).

#### 4.2.5. Effect on Freeze-thaw stability

The freeze-thaw stability for R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> was found to be 2.22-44.21 %, 1.47-39.29 %, 5-55.66 %, and 3.22-51.36 % during the storage period from 0 h to 96 h as shown in Table 7. The freeze-thaw stability increases with the time of storage but it reduced with the dose of irradiation. Similar results for other starches were reported by [39,13,27]. Lower syneresis was exhibited by R<sub>1</sub> and T<sub>1</sub> than R<sub>0</sub> and T<sub>0</sub>; these findings suggested higher structural stability on freezing and thawing. After completion in the first cycle, the values gradually decreased for R<sub>1</sub> and T<sub>1</sub> but increased during the frozen storage period. A similar trend was observed rest of the cycles due to the release of water after thawing it could have decoiled or disturbed the chain of starch but due to modification, the stability of starch was improved which may cause a lower



rate of freeze-thaw values. In the production of frozen and chilled foods, it is essential to consider freeze-thaw stability, which measures the volume of water expelled from the gels during storage due to syneresis. During multiple cycles of freezing and thawing, the culinary banana starch gel exhibited instability, resulting in a leakage of water content ranging from 24.13 % to 42.58 %. Because of the growing amount of water that separated from the gels during extended storage, banana starch is not suited for frozen goods [42]. These findings demonstrate that meals requiring freezing can be preserved alongside irradiation starches. The enhanced freeze-thaw stability of irradiated starches is explained by the weakening of the hydrogen bonds between the amylose-amylose and amylose-amylopectin chains [43,12].

#### 4.2.6. Effect on Paste Clarity

Table 8 illustrates the light transmission (%) of the starch gels at a chilled temperature of 4°C. The transmittance value was decreased from 0 days to 4 days for R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> ranging from 17.16 to 13.10 %, 18.25 to 14.07 %, 19.42 to 14.11 %, and 21.09 to 15.51 % respectively. It was increased as the dosage increased during the 1 to 4-day storage period. The present data demonstrated that the transmittance value of R<sub>0</sub>, R<sub>1</sub>, T<sub>0</sub>, and T<sub>1</sub> was found to be decreased during the storage period from 1 to 4 days but increased with gamma-dose. This is due to the greater swelling ability of starch allows it to have more light through it instead of being reflected. It happens because starch matrices slightly break down during modification. The difference in turbidity showed in R<sub>0</sub> and T<sub>0</sub> due to the varietal differences as well as the difference in amylose content and granular matrices present in both the samples. Due to the low amylose content and the granular size of starch, these types of properties showed lower turbidity in the starches [44,45]. When starch is treated with gamma-irradiation the starch granules might degrade/ fragmentation of the amylopectin chain and start the formation of a carboxylic group which may help to increase the tendency for water binding caused higher transmittance while T<sub>0</sub> and R<sub>0</sub> starch granules may be compressed and show low retrogradation property as compared to R<sub>1</sub> and T<sub>1</sub> consequently decrease in paste clarity. Paste clarity is an important parameter that is used for food processing like transparent paste which is more suited to salad dressing [46].

**Table 7. Freeze-thaw Stability and Transmittance of native and irradiated banana starch**

Freeze-thaw stability (%)	Samples			
	R <sub>0</sub>	R <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
<b>0 h</b>	2.22±0.03 <sup>b</sup>	1.47±0.03 <sup>a</sup>	5±0.12 <sup>d</sup>	3.22±0.05 <sup>c</sup>
<b>24 h</b>	15.68±0.03 <sup>c</sup>	13.37±0.04 <sup>a</sup>	18.55±0.04 <sup>d</sup>	15.40±0.04 <sup>b</sup>
<b>48 h</b>	29.62±0.06 <sup>b</sup>	27.47±0.07 <sup>a</sup>	33.93±0.04 <sup>d</sup>	31.23±0.05 <sup>c</sup>
<b>72 h</b>	32.26±0.05 <sup>b</sup>	30.18±0.03 <sup>a</sup>	45.47±0.06 <sup>d</sup>	422.43±0.05 <sup>c</sup>
<b>96 h</b>	44.21±0.05 <sup>b</sup>	39.29±0.04 <sup>a</sup>	55.66±0.05 <sup>d</sup>	51.36±0.03 <sup>c</sup>
<b>Transmittance (%)</b>				
<b>Day 1</b>	17.16±0.04 <sup>a</sup>	18.25±0.04 <sup>b</sup>	19.42±0.04 <sup>c</sup>	21.09±0.05 <sup>d</sup>
<b>Day 2</b>	15.43±0.05 <sup>a</sup>	17.64±0.15 <sup>b</sup>	18.58±2.25 <sup>b</sup>	18.91±0.08 <sup>b</sup>
<b>Day 3</b>	14.21±0.04 <sup>a</sup>	16.54±0.03 <sup>c</sup>	15.21±0.04 <sup>b</sup>	17.16±0.05 <sup>d</sup>
<b>Day 4</b>	13.80±0.03 <sup>a</sup>	14.07±0.04 <sup>b</sup>	14.11±0.04 <sup>b</sup>	15.51±0.03 <sup>c</sup>

R<sub>0</sub>= Red Banana, R<sub>1</sub>= Treated Red Banana at 5 kGy dose

T<sub>0</sub>= Green Banana, T<sub>1</sub>= Treated Green Banana at 5 kGy

The turbidity of starch paste is influenced by parameters including granule swelling, leached amylose, amylose length, and chain structure [47]. The retrogradation propensity of starch pastes, indicating that banana starch retrogrades when refrigerated, is responsible for the reduction in transmission. Because culinary banana starch has very low paste clarity, it can be used in food products that don't need to be transparent. The increased light transmission brought on by irradiation (paste clarity) is explained by the reduction of swelling starch granules following gelling, the fragmentation of amylopectin branches, and the creation of carboxylic groups with improved hydrophilicity [46]. Transmittance values decline during

storage due to retrogradation (the re-association of amylose chains) and the high concentrations of amylose and phospholipids. Comparable results were seen for Indian beans, wheat, lotus, and kithul irradiation starches [14,13,47].

#### 4.5.1. Effect on Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was employed to identify the functional groups and evaluate structural changes in the starch samples post-irradiation. The absorption bands and their corresponding functional groups are presented in Table 9, with spectral area and length details illustrated in Figure 1. Broad absorption bands observed at  $3289\text{ cm}^{-1}$ ,  $3298\text{ cm}^{-1}$ , and  $3325\text{ cm}^{-1}$  were attributed to the stretching vibrations of O–H, N–H, and C–H groups, indicating the presence of phenolic, amine, and alkane functionalities. Strong bands around  $2343\text{ cm}^{-1}$ ,  $2356\text{ cm}^{-1}$ ,  $2393\text{ cm}^{-1}$ , and  $2436\text{ cm}^{-1}$  corresponded to asymmetric stretching of  $\text{O}=\text{C}=\text{O}$ , suggesting the presence of carbon dioxide. Notably, medium intensity peaks in the range of  $1600\text{--}1650\text{ cm}^{-1}$  ( $1637$ ,  $1647$ ,  $1634$ , and  $1624\text{ cm}^{-1}$  for T0, R0, T1, and R1 respectively) were indicative of amide ( $\text{C}=\text{O}$ ), conjugated alkene ( $\text{C}=\text{C}$ ), and amine bending (N–H) groups. Peaks between  $1400\text{--}1000\text{ cm}^{-1}$ , particularly at  $1006$  and  $1007\text{ cm}^{-1}$ , were associated with alkene ( $\text{C}=\text{C}$ ), sulfonic ( $\text{S}=\text{O}$ ), and anhydride ( $\text{CO}-\text{O}-\text{CO}$ ) groups, suggesting the presence of sugars such as glucose, fructose, and sucrose. Additional peaks in the fingerprint region ( $1200\text{--}650\text{ cm}^{-1}$ ) were recorded at  $772$ ,  $765$ ,  $647$ , and  $640\text{ cm}^{-1}$ , corresponding to C–H and C–Br stretching, indicative of halo compounds. The comparative spectral analysis revealed that gamma irradiation did not induce any major alterations in the characteristic peaks; however, minor shifts in peak intensity and broadness were observed, especially in samples T1 and R1. These variations suggest partial degradation and modification of carbohydrate chains due to irradiation-induced structural rearrangements. Overall, the presence of eight consistent peaks in the  $4000\text{--}500\text{ cm}^{-1}$  range across all samples confirms the structural integrity of the starches with slight modifications under irradiation.

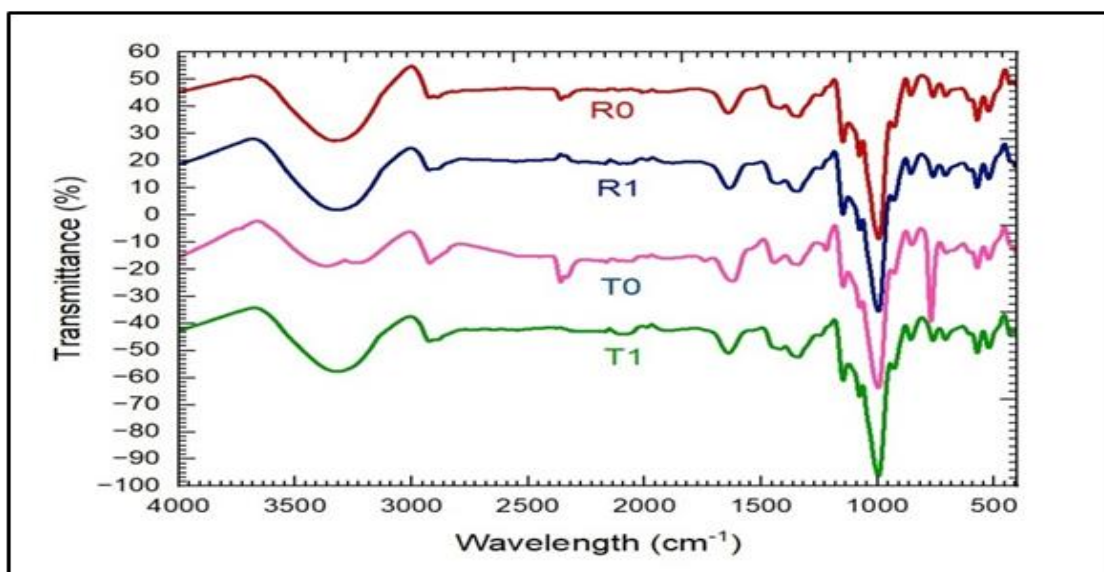


Fig. 1. FTIR combine spectra of native and gamma-irradiated starch

#### 4.5.1. Effect on Scanning Electron Microscope

The banana starch granules from to cultivar had irregular, regular oval and elongated shapes. In T<sub>0</sub> the longer and elongated edges were observed while in R<sub>0</sub>, the oval and irregular shapes were observed. This implies that during isolation process didn't cause damage; or disorganizes the arrangement of starch granules. The shape variation of granules could affect the functional properties which are shown in Fig. 2. In present study showed the major cross-sectional area and length were observed for native starches (R<sub>0</sub> and T<sub>0</sub>) accounting for R<sub>0</sub> area was  $0.0025181\text{ mm}^2$  ( $25181\text{ }\mu\text{m}^2$ ) and the average length was  $53.95\text{ }\mu\text{m}$  and for T<sub>0</sub> area was  $0.5273106\text{ mm}^2$  ( $527310.6\text{ }\mu\text{m}^2$ ) and the average length was  $64.09\text{ }\mu\text{m}$  and average area were found to be R<sub>0</sub> ( $866.4\text{ }\mu\text{m}^2$ ) and T<sub>0</sub> ( $395.3\text{ }\mu\text{m}^2$ ) were shown in Fig. 2.1 and 2.2. After irradiation, the

breakage was observed in starch granules as the T<sub>1</sub> showed elongated and more dense granules with some fissures at the surface whereas R<sub>1</sub> showed irregular shapes with more fissures at the surface of the granules. The major cross-sectional area and length were observed for R<sub>1</sub> and T<sub>1</sub>. The area range for T<sub>1</sub> was 0.00211578 mm<sup>2</sup> (2115.78 μm<sup>2</sup>) and the average length was 18.7 μm and for R<sub>1</sub> the area was 0.0005042489 mm<sup>2</sup> (504.2489 μm<sup>2</sup>) and the average length was 7.5 μm.

**Table 8. The banana starch wave number (cm<sup>-1</sup>) is based on the FTIR spectroscopy profile.**

Wave number (cm <sup>-1</sup> )	Functional Group	Associated Biomolecules	Samples	Interpretation
~3300	O–H stretching	Moisture, cellulose, polyphenols	R0, R1, T0, T1	Broad peak; intensity reduced in R1, T1 indicating moisture loss and dehydration
~2920	C–H stretching	Alkanes (lipids, polysaccharides)	R0, R1, T0, T1	Peak retained; slight shift reflects lipid structure alteration due to treatment
~1640	C=O stretching / Amide I	Proteins, pectins	R0, R1, T0, T1	Minor shift; protein denaturation or bond weakening during processing
~1450	CH <sub>2</sub> bending	Cell wall components (pectin)	R1, T1	Emerges in treated samples; cell wall loosening or rearrangement
~1250–1300	C–N stretching	Proteins (amide III)	R0, T0	Weaker or absent in R1, T1; indicates protein degradation or denaturation
~1025–1050	C–O stretching	Cellulose, hemicellulose, starch	R0, R1, T0, T1	Reduced intensity post-treatment, suggesting partial breakdown of carbohydrates
~875–890	C–H out-of-plane bending	Aromatic compounds	R0, R1, T0, T1	Slightly visible; structural aromatic components retained
~520–600	Fingerprint region	Complex structural vibrations	R1, T1	Strong in treated samples; indicates major structural or chemical changes

#### 4.5.2. Effect on X-ray diffraction

The crystallinity of starch is directly affected by amylose concentration; a lower amylose level results in increased crystallinity, but longer-chain amylopectin structures exhibit greater stability in their crystalline state. The crystalline portion of the starch is produced by the linear structure of amylose, whereas the amorphous phase is caused by the branching structure of amylopectin [49]. A feature of the XRD spectrum before heating confirms the presence of two phases. A wide range of 5° to 30° with a few notable peaks characterize this spectrum (Figure 3). Strong peaks at 2θ values of around 14.8° and 16.9°, a doublet at 22.6°, and weak diffraction peaks at 5.6° and 11.3° were all visible in T1. The weak diffraction peaks at 15.2° and 23.4°, and the strong diffraction peaks at 2θ of 15.4° and a doublet at 17.4°, were indicative of T0, in contrast. Strong diffraction peaks at 2θ values of 15.5°, 17.5°, and a doublet at 19.4° and 23.7° were seen in the R1, along with faint peaks at 5.1° and 15.51°. In contrast, the R<sub>0</sub> presented a pattern with significant peaks at approximately 15.2° and 17.0°, and a doublet at 24.8°, with weak diffraction peaks at 5.2° and 11.2°. The crystallinity of T<sub>0</sub> and R<sub>1</sub> (native starch) exhibited a reduction in peak intensities in T<sub>1</sub>

and  $R_1$  (gamma-irradiated starch) as a result of the degradation of the amylopectin chain. Comparable findings regarding diminished crystallinity have been documented by [17, 19, 12].

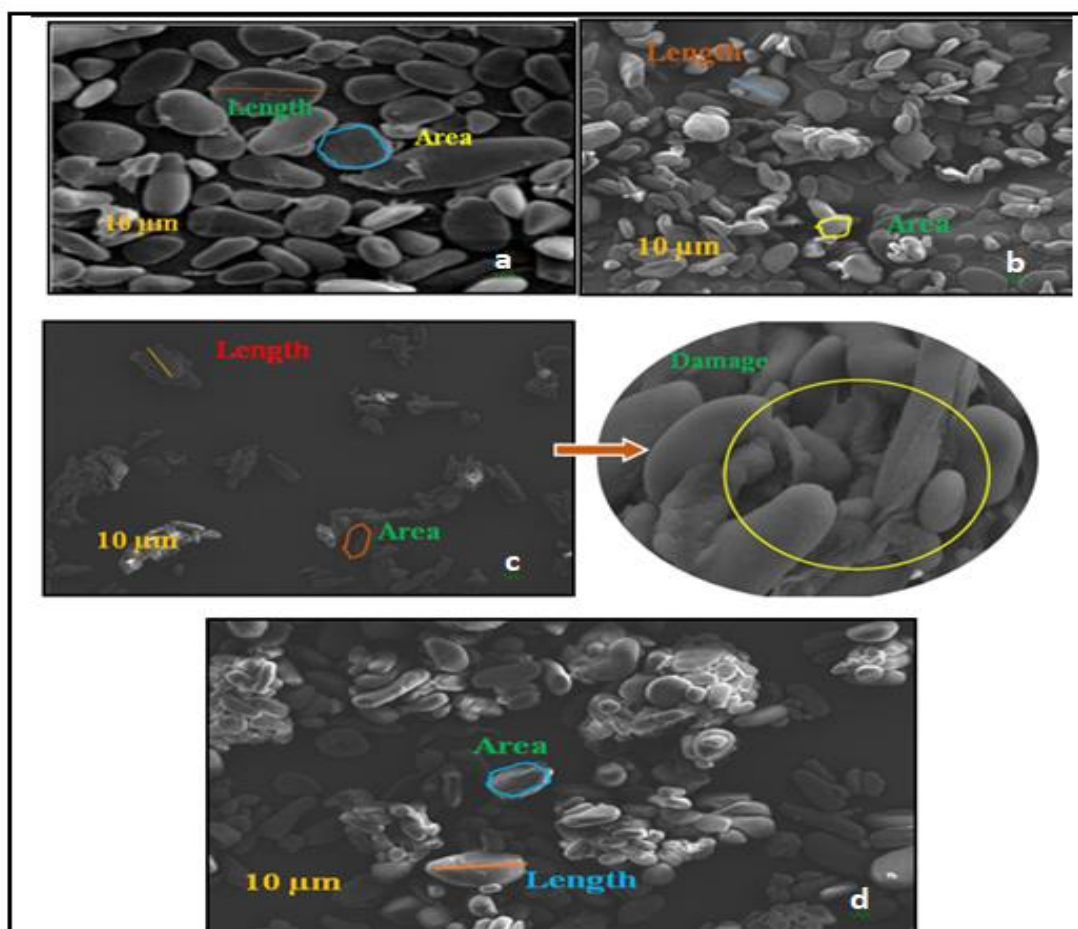


Fig. 2. SEM of native and irradiated banana starches (a)  $R_0$ , (b)  $T_0$ , (c)  $T_1$ , (d)  $R_1$

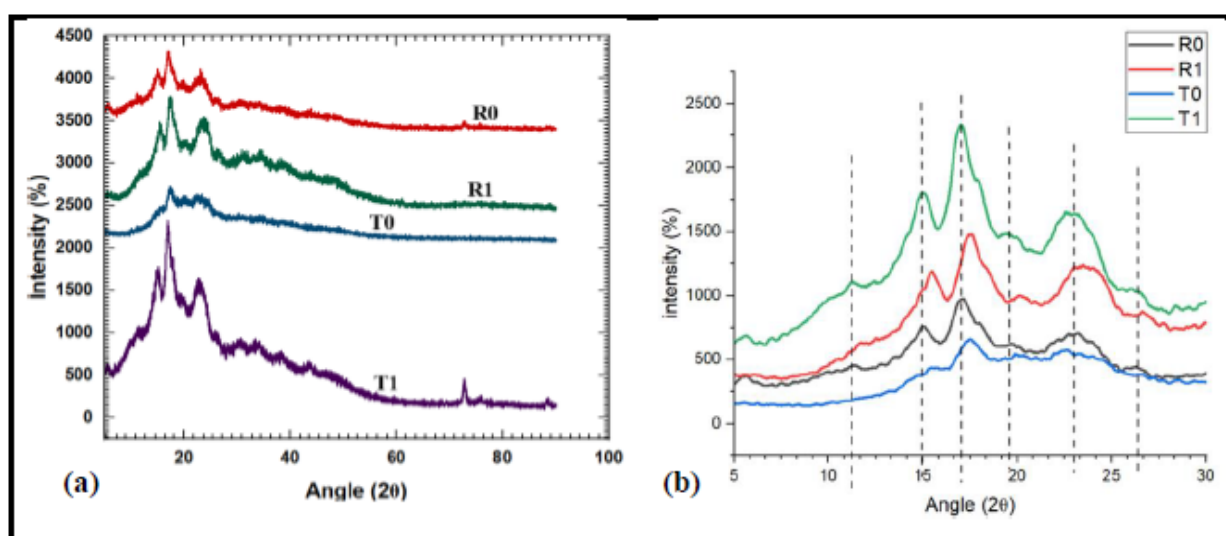


Fig. 3. The diffractogram of  $R_0$ ,  $R_1$ ,  $T_0$  and  $T_1$  starch (a), and the simulation of the main peaks (b) The graphs were separated on the vertical axis by 300 arb unit for better visualization.

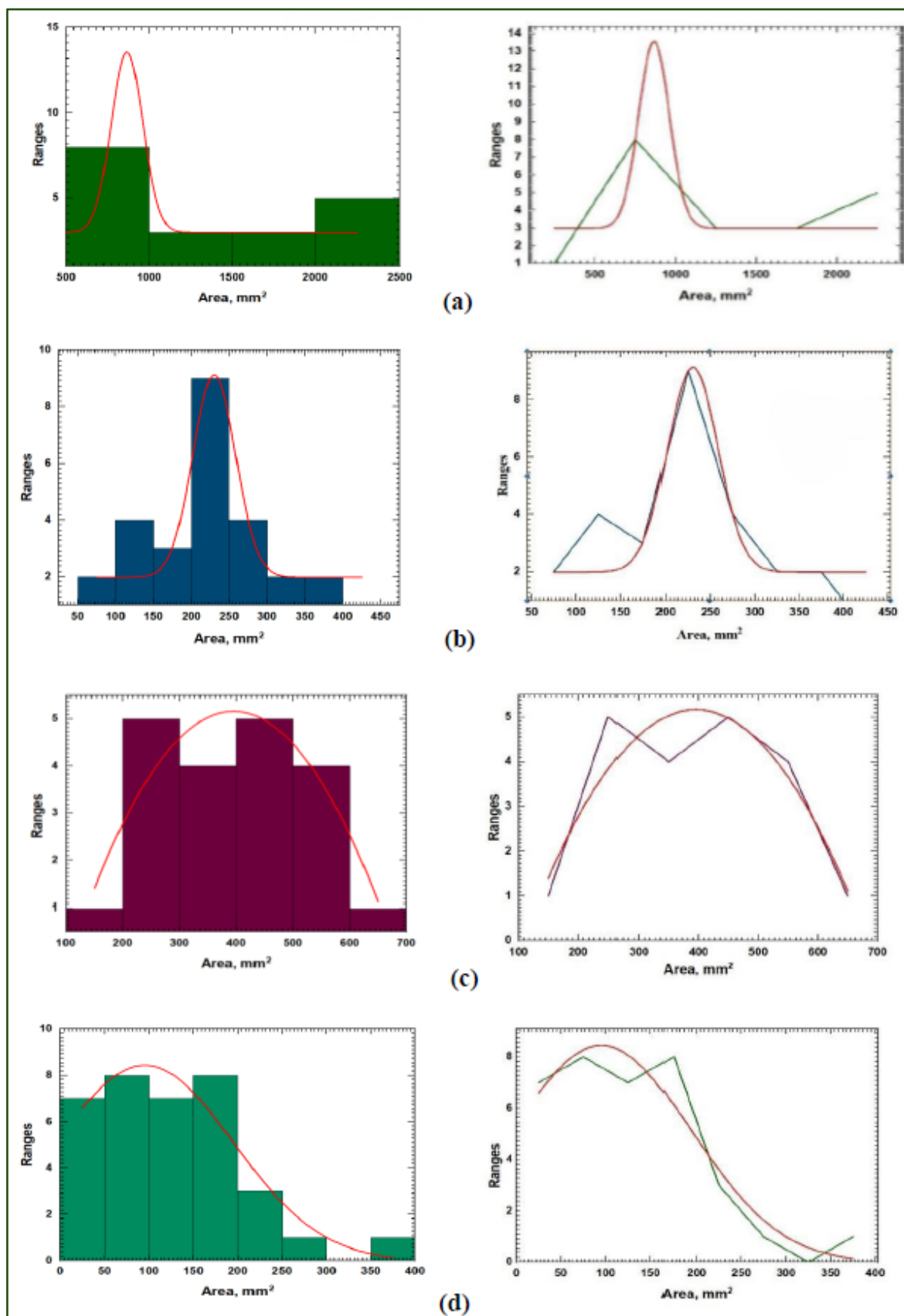


Fig. 2.1. Histogram and line graph distribution of granules cross-sectional area of native and irradiated banana starch (a) R<sub>0</sub>, (b) R<sub>1</sub>, (c) T<sub>0</sub>, and (d) T<sub>1</sub>.



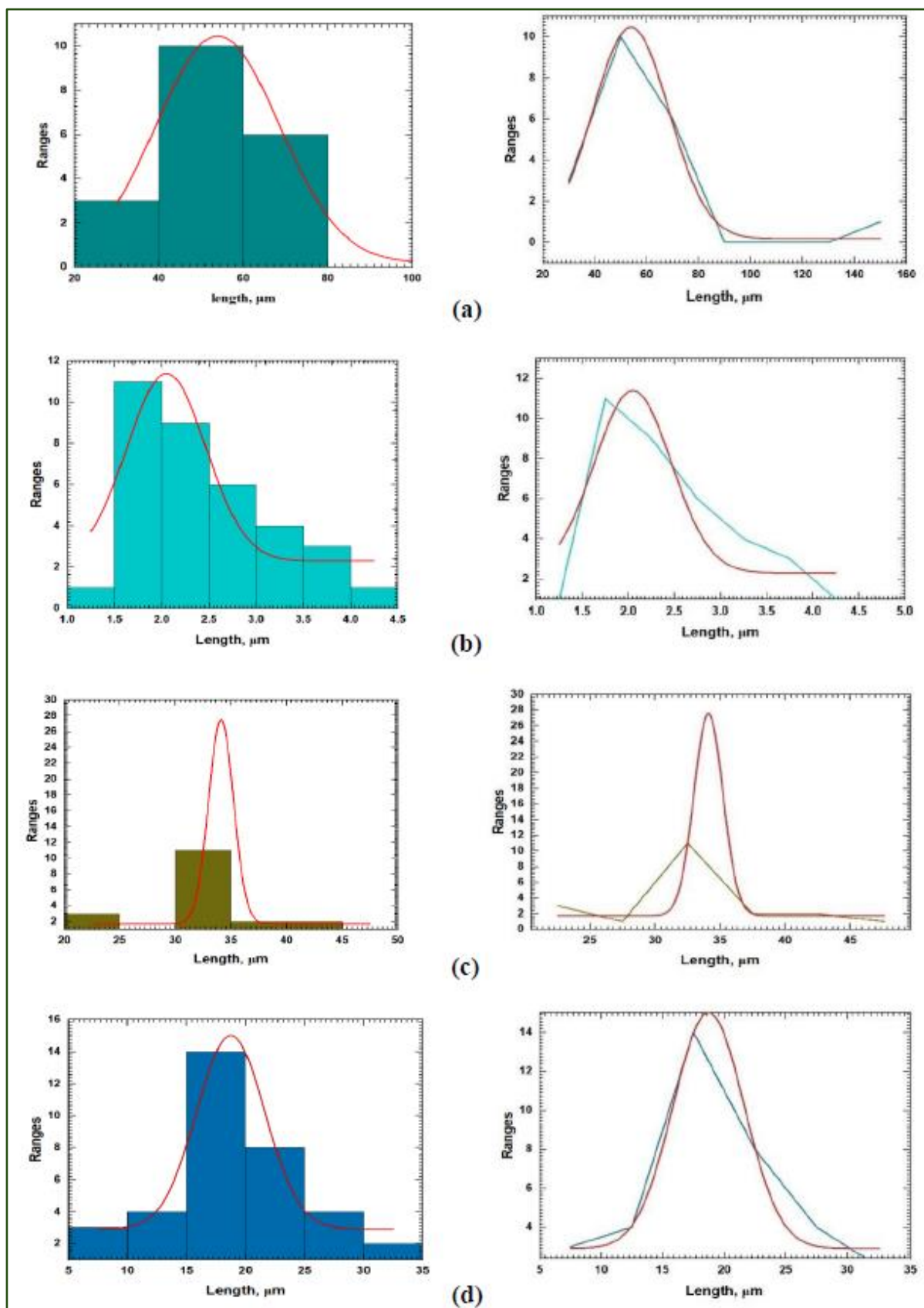


Fig. 2.2. Histogram and line graph distribution of granule length of native & irradiated banana starch (a)  $R_0$ , (b)  $R_1$ , (c)  $T_0$ , (d)  $T_1$

## CONCLUSION

Starch is a naturally occurring, biodegradable, cheap, and abundant polysaccharide molecule. Starch is an important food and versatile biomaterial used worldwide in a wide variety of industries, including the food, healthcare, textile, chemical, and engineering sectors. Gamma-ray irradiation is a non-thermal protection method. Gamma radiation emitted by  $^{60}\text{Co}$  is widely used to kill insects/organisms and improve microbiological safety and food preservation. In the present situation, as youngsters have gluten intolerance, the  $\gamma$ -irradiated banana starch product is a better choice to eat than the commercial product. In the future, it would be advised for those with celiac disease. In general, modified starch is used for the binding and processing of fatty foods and foods, meat products and starch-based snacks, and products made in ovens as puff pastry coatings for fried snacks; in ice cream and salad dressing.

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