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Turmeric Sprout Inhibition and Rhizomes Quality after Post-Harvest Treatment with Gamma Irradiation

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ABSTRACT

A study on sprout inhibition by gamma irradiation in a fresh local variety of turmeric of was carried out. Fresh turmeric was subjected to 0 and 200 Gy and stored at ambient temperature (33.0 ± 1.0°C), 52.5% to 66.5% RH and analyzed after 5, 30, 60 and 90 days in storage. The parameters observed were physiological weight loss, sprouting, color characteristics, total phenolic content, antioxidant activity and curcumin content. The results suggest that a dose of 200 Gy effectively inhibits sprouting in turmeric when compared to the non-irradiated samples after 30 days; moreover, there was no sprouting up to 90 days of storage. The dose of 200 Gy did not affect the levels of total phenolic content, DPPH scavenging activities, ferric reducing antioxidant potentials (FRAP) or curcumin content of the turmeric rhizome. However, these values were altered with storage time. The levels of the total phenolics, DPPH, FRAP and curcumin content of both the non-irradiated and irradiated samples were 7.05 ± 0.48 to 10.14 ± 0.84 mg gallic acid equivalent/g, 3.43 ± 0.29 to 4.62 ± 0.23 mg ascorbic acid equivalent/g, 61.83 ± 1.12 to 69.45 ± 1.91 µmol $FeSO_4$/g and 5.74 ± 0.15 to 11.20 ± 0.85 mg/g of samples, respectively. Therefore, gamma radiation at 200 Gy was found to be an effective post-harvest treatment for sprout inhibition and extension of shelf life of fresh turmeric rhizomes for longer than 60 days without affecting curcumin content and antioxidant activity.

Keywords: Antioxidant activity; Curcumin; Gamma Irradiation; Sprout Inhibition; Turmeric

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1. Introduction

Curcuma longa L. (Zingiberaceae), commonly known as turmeric, belongs to the group of ancient traditional herbal medicines used as a treatment for inflammatory conditions, widely found in India, China and Southeast Asian countries. Rhizomes of this plant have been used as an active drug for the prevention of chronic degenerative disorders. Turmeric constituents include members of a group of bioactive compounds called curcuminoids. The major constituent: curcumin (C), also called diferuloylmethane, is the main bioactive compound, containing natural lipophilic polyphenol found in the rhizome of *Curcuma longa* (60-70%); this molecule imparts the brilliant yellow color seen in *Curcuma spp*. [1]. Almost all of the pharmacological activities of *Curcuma spp.*, including antioxidant, anti-inflammatory activities, antimicrobial properties, anti-carcinogenic activities and toxicological activities, have been contributed to curcumin. The other curcuminoids include desmethoxycurcumin (DMC) (20-30%) and bisdemethoxycurcumin (BDMC) (10-15%), which also exhibit pharmacological activities like curcumin but are present in lower concentrations [2].

The number of pharmaceutical companies developing herbal preparations has grown in recent years [3]. Storage of fresh turmeric for local natural food supplements, cosmetic products or herbal medicine is very important, as fresh turmeric is only available for a few months of the year. Ionizing irradiation treatments, such as gamma rays, X-rays or electron beam, have emerged as the preferred methods for food irradiation. Appropriate use of irradiation can extend the shelf life of foods and agricultural products. A great number of studies in several countries have reported that irradiation of tubers, such as potato, onion, garlic, and sugar beet, with a low dose of gamma radiation inhibits sprouting and reduces weight loss of bulbs [3-4]. Therefore, the application of irradiation may be an effective post-harvest treatment for controlling undesirable changes in tubers and bulbs during long term storage. However, few studies have been conducted on how irradiation treatment may impact the potentially important phytochemicals found in the fresh tubers and herbal rhizomes.

The objectives of this study were to determine the effects of gamma irradiation on sprouting inhibition and shelf- life extension in turmeric rhizomes at high temperature storage conditions, to find a sufficient dose to increase the shelf life of fresh turmeric rhizomes without affecting their quality and phytochemical activities.

2. Materials and Methods

2.1 Plant materials

The fresh turmeric (*Curcuma longa* L.) of a local variety used in this study was obtained from Kanchanaburi province, in the western region of Thailand. They were harvested in March 2019 (9-10 months after planting). The rhizomes (10 days after being harvested) were washed, brushed and dried at ambient temperature for 7 days. Fresh rhizomes within a standard weight range were chosen randomly in six batches. The quantities of approximately 1.70 kg for each sample were placed in aerated boxes measuring 24 x 40 x 12 cm. Six packets of each treatment were performed, totaling 12 packets at 20.4 kg, and were stored at room temperature (25^0C) for 18 hours before irradiation. Samples was transferred and exposed to gamma radiation at an average dose of 200 Gy. The non-irradiated samples served as control.

2.2 Gamma irradiation treatment

Fresh turmeric rhizomes were subjected to gamma irradiation via a Cobalt-60 source with an IC multipurpose irradiator at the Irradiation Center, Thailand Institute of Nuclear Technology (Public Organization), Thailand. The gamma irradiation dosage was determined on the basis of dose per time rates (71.61 G/ hr). Rhizomes were surrounded

with alanine dosimeters to verify irradiation exposure using a paramagnetic resonance spectrophotometer. The minimum adsorbed dosage was 171.42 Gy and maximum adsorbed dosage was 224.57 Gy, while the average dosage measured was 198 Gy.

2.3 Storage treatment

After irradiation, rhizomes were stored at 33.0 ± 1.0^{0}C at 59.5 ± 7.0% relative humidity for up to 90 days. At 5, 30, 60 and 90 days after storage, interval samples were collected and evaluated for the presence of sprouts, weight loss, color characteristics and analyzed of curcumin content, total phenolic content, antioxidant activities, such as ferric reducing antioxidant potentials and DPPH scavenging activities.

2.4 Sprouts and weight loss

Samples were removed from the storage room after 5, 30, 60 and 90 days and the sprouts from the sprout rhizomes were collected, weighed and expressed as gr/1.7 kg. The total sprout weight was determined and expressed as mean ± SD and calculated as percentage of sprout weight compared with all total rhizomes, measurements were done in triplicate. Final rhizome weight after storage minus sprout was subtracted from the initial weight prior to storage then the weight loss was determined with the following formula.

$$\%\text{sprout wt} = \frac{\text{Sprout weight} \times 100}{\text{Total weight of rhizomes}},$$

$$\%\text{wt loss} = \frac{\left(\text{Initial weight - final weight}\right)}{\text{Initial weight}} \times 100\%.$$

2.5 Color characteristics

Samples of 250 grams from each treatment group were collected at 5, 30, 60 and 90 days of storage. The samples were measured for color in Hunter *L a b* color system by Hunter *L a b* colorimeter (Chroma Meter Konica Minolta CR- 300, Osaka, Japan), the standard light source was D65. Each sample was measured in 20 replicates. The data were analyzed by analysis of variance (ANOVA) to compare between groups. The non-uniformity of ΔE was calculated and obtained from the average original color (5 days after storage) and the average value of *L a b* at 60 days of storage using the following formula.

$$\Delta E = \sqrt{DL^2 + Da^2 + Db^2}.$$

ΔE of irradiated samples and control samples were compared with paired t- test with a 95% confidence level for determining efficacy of storage time and irradiation level on color characteristics of turmeric.

2.6 Preparation of extracts

Two hundred grams of turmeric rhizomes were collected from 3 replicates of the batches at different intervals during the storage period, cleaned, washed and air dried. All rhizomes were homogenized with a blender. Then, 25 g of the samples were suspended in 250 ml of 95% methanol and sat at room temperature for 24 hrs.

2.7 Quantification of curcumin content

The curcumin content of the extract was analyzed using HPLC. The HPLC separation of curcumin was performed with a C_{18} column (Phenomenex, USA) (250x4. 6 mm, Jasco, Japan). The chromatograms of samples were produced using a UV detector at 425 nm. The mobile phase was 85% ethanol with a flow rate of 0.8 ml/min. The content of curcumin was determined and calculated using a calibration curve of standard curcumin (Sigma, USA).

2.8 Total phenolic content

The total phenolic content (TPC) of the extract was estimated using the Folin-Ciocalteu assay following a previously published method [5]. Results are expressed as mg gallic acid equivalent (GAE)/g of sample.

2.9 Free radical scavenging activities with DPPH

The DPPH (1-1-Diphenyl-2-picryl hydrazyl) assay was used to evaluate anti-oxidative activity. The assay method used was modified from that described in a previous report [6]. Activity is expressed as mg ascorbic acid equivalent (AAE)/g of sample.

2.10 Ferric reducing antioxidant potentials

The ferric reducing antioxidant potential (FRAP) assay was performed using a method of reduction ferric ions in order to form ferrous tripyridyltriazine (TPTZ) complex [7]. Ferric reducing ability is expressed as μmol/$FeSO_4$/g.

2.11 Statistical analysis

The data was collected for non-irradiated and irradiated rhizomes, resulting in 3 replicates for each treatment. ANOVA was performed with SPSS software and the differences among treatment means were determined by Duncan's new multiple range test.

3. Results and Discussion

3.1 Sprout inhibition and weight loss

The results in this study showed that gamma irradiation at doses of 200 Gy at 17 days after harvest for turmeric rhizomes kept at high temperature (33.0 ± 1.0^0C, 59.5 ± 7.0% RH) could completely prevent sprouting after storage for a time of 90 days (Table 1). Non-irradiated samples showed a significantly higher level of sprouting and weight loss, increasing with storage time at 30, 60 and 90 days, whereas irradiated samples were found to be effective in sprout inhibition and had decreased weight loss at all storage times. Many studies have indicated that irradiation during the dormancy period of tubers is the most effective for sprout control [8]. Rhizomes in the early stage after harvest are in a metabolically active state and are therefore more sensitive to irradiation, which can affect the hormonal synthesizing system and nucleic acids, and therefore fail to sprout. On the other hand, higher storage temperatures require higher doses of radiation [9]. Other studies have indicated that the high respiration rate of rhizomes in the early stage after harvesting have increased membrane permeability and may have increased weight loss [10]. The greater weight loss (59.64%) in the non-irradiated samples stored at 90 days may be have been due to a higher respiration rate, increased membrane permeability and more sprout development. The lower weight loss seen in the irradiated samples (56.46%) may have been due to a delay in wound healing and a change in the membrane function of the irradiated rhizomes [11-12].

3.2 Color Characteristics

The irradiated turmeric rhizomes were measured for their color values in Hunter *L a b* color system as shown in Table 2. Colors are considered to be one of the most significant properties of turmeric, where poor quality rhizomes have low customer acceptance. In regards to lightness (*L* value), the differences in color measurements between the non-irradiated and irradiated samples at 5, 30 and 60 days of storage were considered non-significant. However, a significant increase in the L *value* of non-irradiated samples was observed at 90 days. A similar effect was also found for redness (*a* parameter) and yellow (*b* parameter), whereas the differences between non-irradiated and irradiated samples at storage time intervals of 5 to 90 days were found to be non- significant. The non-uniformity of ΔE was used to determine overall changes in fresh rhizomes. If the non-uniformity of ΔE was found to be greater than 1, the two colors are considered completely different. The results showed that non-uniformity of ΔE for non-irradiated and 200 Gy irradiated rhizomes was greater than 1 at 60 days of storage (Table 3). It appears that storage time

has an effect on the rhizomes, which differs from the original color. This finding suggests that the color characteristics of rhizomes were affected by both storage time and weight loss. Many studies have suggested that changes observed in the color of irradiated foods were related to the creation of new color compounds through either the Maillard reaction, non- enzymatic browning, or the systematic oxidation of phenolic compounds present in the food matrix [13-14].

3.3 Curcumin content

The quantification of curcumin from extracts of non-irradiated and irradiated turmeric rhizomes are shown in Table 4. Gamma irradiation at a dose of 200 Gy did not affect the curcumin content of the turmeric extract studied. Non-irradiated and irradiated treatments from all storage times (5 to 90 days) after 23 days of post- harvest irradiation did not result in a significant change in curcumin content. However, a curcumin content was found to increase with increasing storage time. Some studies have reported that irradiation at certain doses can enhance bioactive components. Dhanya et al. [15] reported that gamma irradiation at 1, 3 and 5 kGy of fresh peeled turmeric rhizomes slightly expanded the curcuminoid content due to the gamma radiation induced damaging of cell membranes, resulting in an increased extractability of active compounds, while 5 kGy was found to be the optimal dose for microbial decontamination and maintaining the quality of rhizomes.

3.4 TPC, FRAP and DPPH activities

The results showed that turmeric rhizomes irradiated at 200 Gy did not show any significant difference in TPC, FRAP or free radicals scavenging with DPPH (Table 5) when compared to the control samples at all storage times. However, after 60 days, TPC, FRAP and DPPH significantly increased in samples from both groups. The results revealed that curcumin content, TPC, FRAP and DPPH were not affected by irradiation; they varied with storage time and were simply the result of continuous water loss during storage, evidenced by the increased weight loss over time, at all storage times at high ambient temperatures. The highest curcumin content, TPC, FRAP and DPPH values were found at 90 days of storage. In other studies, Wu et al. (1994) reported gamma irradiation at doses of 50 Gy inhibited sprouting with no difference in acceptance test of triangle test between irradiated and non- irradiated ginger (*Zingiber officinale* Roscoe) stored at ambient temperature for 30 days. However, significantly lower levels of some major volatile compounds were found in irradiated rhizomes compared to non-irradiated rhizomes after 3 months of storage [16]. Studies conducted in many countries report that doses between 50 and 150 Gy are recommended for sprout control of tubers during the dormant state shortly after harvest, but optimal treatment depends on the specific variety in question. In this study, the 200 Gy dose of gamma radiation could extend shelf life, inhibit sprouting and decrease weight loss in fresh turmeric rhizomes stored at high ambient temperatures without any effects on bioactive compounds and phytochemical activities.

Table 1. Percentage of sprout inhibition and weight loss on irradiated turmeric rhizomes.

Parameters	Doses (Gy)	Storage time (days)			
		5	30	60	90
Sprout weight *(%)*	0	0±0.00^{a}	47.73±5.33^{b}	59.06±5.08^{c}	63.69±4.27^{d}
	200	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
Weight loss *(%)*	0	0.0±0.0^{1a}	27.82±1.22^{b}	39.49±2.76^{c}	59.64±1.62^{e}
	200	0.0±0.0^{1a}	27.02±1.38^{b}	37.09±1.36^{c}	56.46±0.24^{d}

The different letters within the row and the column indicate the significant difference at 95% confidence level.

Table 2. Color characteristics in Hunter *L a b* system of irradiated turmeric rhizomes.

Color	Doses (Gy)	Storage time (days)			
		5	30	60	90
L	0	51.20±3.10^{a}	51.54±3.05^{a}	52.01±2.09^{a}	53.76±3.51^{b}
	200	51.10±1.79^{a}	52.48±0.68^{ab}	51.78±1.80^{a}	51.10±2.06^{a}
a	0	28.12±5.65^{abc}	26.60±1.88^{ab}	26.12±4.24^{ab}	25.71±6.26^{a}
	200	30.28±5.20^{c}	28.01±3.37^{abc}	28.98±3.73^{abc}	28.97±3.59^{bc}
b	0	30.75±2.34^{abc}	30.51±1.98^{abc}	30.01±1.52^{a}	31.29±2.48^{bc}
	200	30.51±1.31^{abc}	31.54±0.64^{c}	30.25±1.90^{ab}	30.33±1.18^{abc}

The different letters within the rows and the columns indicate the significant difference at 95% confidence level.

Table 3. Calculated ΔE of irradiated turmeric rhizomes at 60 days of storage.

Doses (Gy)	Non- uniformity (ΔE)
0	3.11±1.07
200	3.52±1.74

Table 4. Curcumin content, total phenolic content, ferric antioxidant potential (FRAP), free radicals scavenging with DPPH (DPPH) and of irradiated turmeric rhizomes.

Parameters	Doses (Gy)	Storage time (days)			
		5	30	60	90
Curcumin	0	5.79±0.21^{a}	6.97±0.12^{b}	10.63±0.54^{c}	11.13±0.54^{c}
*(*mg/g*)*	200	5.74±0.15^{a}	7.91±0.41^{b}	10.52±0.32^{c}	11.20±0.85^{c}
Total phenolic content	0	7.05±0.48^{a}	7.28±0.59^{a}	9.35±1.17^{b}	9.92±1.71^{b}
*(*mg GAE/g*)*	200	7.35±0.20^{a}	7.32±0.50^{a}	10.11±0.50^{b}	10.14±0.84^{b}
FRAP	0	61.83±1.12^{a}	60.44±2.97^{a}	67.79±1.64^{bc}	69.45±1.93^{c}
*(*μmol $FeSO_4$/g*)*	200	60.11±2.14^{a}	59.74±3.22^{a}	65.50±0.93^{b}	68.22±1.26^{bc}
DPPH	0	3.56±0.26^{a}	3.64±0.21^{a}	4.08±0.16^{bc}	4.45±0.08^{cd}
*(*mg AAE/g*)*	200	3.43±0.29^{a}	3.74±0.27^{ab}	4.09±0.11^{bc}	4.62±0.23^{d}

The different letters within the row and the column indicate the significant difference at 95% confidence level.

4. Conclusion

Turmeric rhizomes (*Curcuma longa* L.), grown in the western region of Thailand, were assessed after gamma irradiation and prolonged storage for sprout inhibition. The results indicated significant effects of irradiation on sprout inhibition and weight loss. Fresh rhizomes displayed no sprouting when irradiated at 200 Gy and stored at 33.0 ± 1.0^0C, $59.5 \pm 7.0\%$ RH for 90 days. Extensive sprouting was found in non-irradiated rhizomes when stored for 30 days. Curcumin content and antioxidant activity level decreased with increasing storage time for both the irradiated and non-irradiated rhizomes. The most suitable storage time for irradiated rhizomes was found to be no longer than 60 days, due to the severity of shriveling that results from extensive weight loss.

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