Review Article

Journal of Nuclear Technology in Applied Science

Year 2024, Volume-12, Issue-1 (January - December)

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Improvement of the Shelf-Life of *Chrysophyllum Albidum* (African Star Apple) Using Gamma Irradiation

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ARTICLE INFO

Key words: *Food Irradiation, Shelf life, Gamma dose.*

doi: 10.48165/jntas.2024.12.1

ABSTRACT

Chrysophyllum albidum is native to tropical Africa. This seasonal plant has been found to contain more vitamin C than most fruits. In this study, gamma irradiation which offers an option of disinfecting and elongating the shelf life of fruits will be employed to improve the shelf life of *C.albidum*.

Pest free fruits plucked at maturity were washed with brine and cleaned with ethanol. Four sets of fruits were irradiated with different gamma doses: 0, 250, 300 and 600 Gy. Moisture, Phenol, flavonoid and ascorbic acid content were determined for the various batches at weekly intervals. DPPH radical scavenging activities, total heterotrophic count, E. coli count, coliform count and fungal count were also determined.

The percentage of the fruit that got spoilt with 300 Gy are 4.5%, 21.43% and 66.67% for week one, two and three respectively. The moisture content of all the batches ranged from 51.3 to 58.2%. Fresh sample of *C.albidum* has similar DPPH Radical Scavenging percentage inhibition as ascorbic acid. The flavonoid, phenol and ascorbic acid concentrations were 3.716, 1.107 and 1.585 mg/g respectively. The result showed almost no growth in total heterotrophic count, E. coli count, coliform count and fungal count for most of the irradiated samples.

Introduction

The *Chrysophyllum albidum* (African Star Apple in English, Agbalumo in Yoruba and Udala in Igbo) is a native of many parts of tropical Africa. It features prominently in the compound agroforestry system for fruit, food, cash income and other auxiliary uses including environmental uses (Kang, 1992). The tree grows as a wild plant and belongs to the family of Sapotaceae which has up to 800 species and make up almost half of the order (Ehiagbonare *et al*., 2008). The plant has in recent times become a crop of commercial value in Nigeria (Oboh *et al*., 2009). *Chrysophyllum albidum* is a fruit of African origin that is very rich in vitamin C and anti-oxidant effects. It has been found to contain 10 times more vitamin C than orange, banana, paw-paw, etc. (Ige and Gbadamosi, 2007).

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Received 25.09.2024; Accepted 06.10.2024

Presently the only preservation process to extend the shelf life is through refrigeration which extends the shelf life by some days. Food irradiation is a new technology that can increase both the safety and shelf life of food. The use of gamma irradiation to disinfest and preserve fruit has been on-going for more than 40 years (Follett, 2001). In this study, gamma irradiation in addition to refrigeration will be employed to improve the shelf life of *Chrysophyllum albidum*.

Most fruits from Nigeria are not exported because of their short shelf life making it very necessary to improve the shelf lives of these fruits as a means of generating more employment opportunities and exports. Very few works have been reported on elongating the shelf life of *C. albidum*, with most focusing on refrigeration. Food irradiation offers an option of disinfecting and elongating the shelf life of fruits with the aim of making them available all seasons. Nigeria, in trying to boost food security recently purchased a food irradiator, stationed at Sheda Science and Technology Complex (SHESTCO), Abuja; and this has fuelled research into the use of irradiation in fruit preservation.

Methodology

Sample Collection

C. albidum fruits (see figure 1) were collected at a farm in Ido town, Ido local government area with coordinates 7^0 27' 50.99"N, $3^0$44'24.7164"E. The fruits were collected at three intervals. The microbiologist was consulted to guide in the sample collection. Pest free fruits were plucked at maturity to ensure best quality.

Fig. 1: *C. albidum* fruits on the tree

Sample Preparation

The *C. albidum* fruits were washed with brine and cleaned with ethanol to remove the sap and any contamination from the fruits. After the cleaning, great care was taken not to introduce contamination. Four sets of twenty four pieces of fruits were packed into Ziploc bags and tightly taped to make it airtight (see figure 2). They were kept inside the room to be away from insects.

Fig. 2: Packed *C. albidum* fruits

Sample irradiation

Samples were irradiated in three batches of twenty four pieces. The fourth batch was not irradiated to serve as control. They were irradiated with different gamma doses: 250, 300 and600 Gy. This was done to help us to determine the optimum dose which is a balance between what is needed and what can be tolerated by the products (EFSA, 2011).

Physical observation

The mass was measured before irradiation and after irradiation. The moisture content was determined after irradiation using ASAE standard S352.2 (ASAE, 1998). Randomly selected fruits were cut into pieces and weighed using electronic digital balance. The cut pieces were oven dried to constant weight at 150°C (Fig 3)

The weights of sample before drying (W1) and after drying (W2) were recorded. The moisture content (MC) was calculated using the equation below.

$$MC = \frac{(W_1 - W_2)}{W_1} \times 100\% \qquad 1$$

Fig. 3: Drying the fruits.

Chemical Analysis

Five batches of four fruits were sent to pharmacological chemistry laboratory of the University of Ibadan for chemical analysis. Phenol content, flavonoid content, ascorbic acid content and DPPH radical scavenging activities were determined for the various batches.

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.

Determination of Total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Assay of ascorbic acid (by Iodimetry)

Ascorbic acid Determination Content (British Pharmacopeia 1986)

This assay was carried out with slight modification.

About 2g of the sample was weighed using an Analytic weighing balance and soaked in 50ml of distilled water (since ascorbic acid is soluble in water) for about 3hrs. The sample was later filtered using Whatman filter paper no 11cm. 25ml of 1M H_2SO_4 was added to the filtrate followed by the addition of 3ml of starch Mucilage solution.

The Solution was titrated against 0.05M iodine solution until a persistent violet-blue colour is obtained. The percentage w/v of Ascorbic acid in a given sample is calculated.

DPPH scavenging activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to Manzocco et al., 1998 with slight modifications the sample extract (1 mL) is diluted with 1mL of DPPH solution (0.3mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below in equation 2.

$$\%\,\text{inhibition of DPPH radical} = \frac{\text{Abr} - \text{Aar}}{\text{Abr}} \times 100 \qquad 2$$

where Abr is the absorbance control and Aar is the absorbance of the sample reaction has taken place.

Microbiological Analysis

Sample collection

Samples of *Chrysophyllum albidum* were periodically brought into the Microbiology laboratory for microbiological analysis for a period of four weeks. The samples were analyzed within one hour of collection/submission.

Media preparation

The culture media used included nutrient agar, Eosin Methylene blue, MacConkey agar and Potato dextrose agar for the determination of the total heterotrophic count (THC), E. coli count, coliform count and fungal count respectively (see table 1).

Table 1: Culture medium and organisms targeted

CULTURE MEDIUM	ORGANISMS TARGETED
Plate Count agar	Total Heterotrophic Count (THC)
MacConkey agar	Total Coliform Count (TCC)
Eosin Methylene Blue	Total *E. coli* Count (TEC)
Potato Dextrose Agar	Total Fungal Count (TFC)

Enumeration of the microbial load of Chrysophyllum albidum samples

The samples were teased and mashed in alcohol-sterilized laboratory pestle and mortar and a stock solution was made by dissolving 1g of the mashed sample in 10mL of sterile peptone water. The enumeration of the bacterial load in the samples was done using the pour plate technique (Harrigan and MacCance, 1976). The samples were serially diluted using peptone water as the diluent. Aliquots of the selected dilutions were plated out on the appropriate medium and incubated. The plates were observed after the incubation period and observations were noted and recorded. Presumptive bacteria were selected and repeatedly sub-cultured to obtain pure cultures, and few of the bacteria were selected for identification using sugar fermentation and biochemical characters (Sneath, 1996).

Identification of the fungi

The characterization of the fungi was carried out using morphological and microscopic techniques (Lactophenol Blue staining technique).

Result

Physical observation

The mass measurements are given in table 2. There was slight reduction in the weight of the *C. albidum* after irradiation. The percentage reduction ranged from 0.04 to 2.46 %. This reduction increased with increasing dose. The moisture content analysis is given in table 3. The moisture content ranged from 51.3 to 59.5 %. Again the reduction increased with increasing dose.

From the physical observation, the fruits were changing colour and getting bad. The percentage of those that got bad is tabulated in table 4. For the first week the percentage that got spoilt ranged from 4.55 to 36.36 %. In the

Table 2: Weight of C. *albidum* fruits

DOSE	DATE	MASS		
		BEFORE IRRADIATION	AFTER IRRADIATION	PERCENTAGE REDUCTION
0 Gy	12/02/2019	1070.6 g	NA	NA
250 Gy	21/02/2019	1306.2 g	1305.7 g	0.04%
300 Gy	12/02/2019	1074.6 g	1073.9 g	0.07%
600 Gy	19/02/2019	1331.2 g	1298.3 g	2.46%

Table 3: Moisture content analysis result

DOSE	WET WEIGHT (g)	DRY WEIGHT (g)	% MOISTURE CONTENT
0 Gy	110.8	46.3	58.2
0 Gy (one week)	100.5	40.7	59.5
250 Gy (one week)	98.6	54.9	55.7
300 Gy (one week)	87.0	39.2	54.9
600 Gy (one week)	90.2	46.3	51.3

second week, it ranged from 21.43 to 71.43 %. In the third week, it ranged from 66.67 to 100 %. The set of fruit with 300 Gy had the lowest percentage throughout the weeks while the set with no radiation had the highest percentage of spoilt fruits.

Table 4: Percentage of fruit that got bad

DOSE	WEEK 1	WEEK 2	WEEK 3
0 Gy	36.36 %	71.43 %	100 %
250 Gy	27.27 %	71.43 %	100 %
300 Gy	4.55 %	21.43 %	66.67 %
600 Gy	18.18 %	57.14 %	91.67 %

Chemical Analysis

Table 5 shows us that fresh sample of *C. albidum* has more DPPH Radical Scavenging percentage inhibition than ascorbic acid. Samples that were irradiated with 300 Gy showed better inhibition than those that were not irradiated after one week. Table 6 shows the result of the chemical analysis. After one week, samples with no radiation and that of 300 Gy showed similar flavonoid, phenol and ascorbic acid values as fresh samples. All the samples had similar phenol values as the fresh fruit. All the samples had lower ascorbic acid values than the fresh fruit.

Microbiological Analysis

The results of the bacterial load obtained from the experimental set-up are shown in the following Tables 7 (Total Heterotrophic Count), 8 (Total *E. coli*) and 9 (Coliform count). There was no growth of *E. coli* on any of the samples except on the 600 Gy samples at Week 4. This might be due to the physical damage of the fruits as a result of the high irradiation level and also the condition of storage. The same observation was noted in the coliform level. Most of the samples had no bacterial growth though some of the growth seen in the fourth week maybe due to environmental conditions.

All the samples had little fungal growth as noticed on the culture medium and this could be linked to the contact the fruits had with the soil and other organic matter at the point of harvesting and sampling. Fungi of the genera *Aspergillus* and *Fusarium* were observed on all the samples. With the exception of 600Gy at week 3 and 4, there was no observable growth on other treatments and the control as the experimental duration increased. However there was observable growth on the control and 250 Gy-treated samples by Week 1.

In future studies, cleaning of the samples by washing and alcohol disinfection will be carried out effectively, to prevent external contaminants from influencing the experimental observation.

Table 5: DPPH Radical Scavenging % inhibition of ascorbic acid Standard

Conc.(µg/mL)	%inhibition					
	Ascorbic acid	Fresh sample	Fresh sample one week no radiation	One week 250 Gy radiation	One week 300 Gy radiation	One week 600 Gy radiation
1000.00	94.55	93.95	93.55	89.85	93.75	8.89
800.00	93.95	93.85	93.35	65.12	93.65	3.82
600.00	93.55	93.75	92.66	58.89	86.62	-1.24
400.00	92.76	93.55	89.39	18.14	82.95	-2.31
200.00	77.70	87.11	44.40	3.02	77.40	-3.91

Table 6: Chemical analysis result

Parameters	Fresh (1000 ug/ml)	One week no radiation (1000ug/ml)	One-week 250 Gy radiation (1000ug/ml)	One-week 300 Gy radiation (1000ug/ml)	One-week 600 Gy radiation (1000ug/ml)
Flavonoid (mg/g)	5.846	4.816	-0.454	3.716	-0.584
Phenol (mg/g)	1.286	1.239	1.568	1.107	1.331
Ascorbic acid (mg/g)	4.403	1.761	1.056	1.585	0.880

Table 7: Total Heterotrophic Count (THC) ($\times 10^1$cfu/g)

Sample	Week 1	Week 2	Week 3	Week 4
No Radiation	1.2	NG	NG	NG
250 Gy	0.1	NG	NG	NG
300 Gy	NG	NG	NG	NG
600 Gy	NG	NG	0.3	41.8

Note: Each value is a mean of three replicates.

Table 8: Total E. coli ($\times 10^1$cfu/g)

Sample	Week 1	Week 2	Week 3	Week 4
NoRadiation	NG	NG	NG	NG
250 Gy	NG	NG	NG	NG
300 Gy	NG	NG	NG	NG
600 Gy	NG	NG	NG	27.8

Note: Each value is a mean of three replicates.

Table 9: Coliform count ($\times 10^1$cfu/g)

Sample	Week 1	Week 2	Week 3	Week 4
No Rradiation	NG	NG	NG	NG
250Gy	NG	NG	NG	NG
300 Gy	NG	NG	NG	NG
600 Gy	NG	NG	NG	38.8

Note: Each value is a mean of three replicates.

- NG: No growth on the culture plate after the incubation period

Conclusion

The optimum dose for improving shelf life was found to be around 300 Gy. There might be a need to carry out an analysis of the 600Gy–treated samples to ascertain the cause of the growth noticed on the third and fourth week. Irradiation offers a very reliable way of increasing the shelf life of the fruits, hence could offer another avenue for the preservation of the fruits.

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