Environment and Ecology 41 (3C) : 1917—1922, July—September 2023
Article DOI: https://doi.org/10.60151/envec/TQOJ6134
ISSN 0970-0420

Effect of Various Doses of Gamma Irradiation on the Nutrient Composition of Mahua (*Madhuca indica*) Flower Stored at Ambient Temperature

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Received 31 March 2023, Accepted 20 June 2023, Published on 4 September 2023

ABSTRACT

Mahua flower is an important non-timber forest produce and has a great importance in tribal communities. It is frequently under appreciated due to poor quality brought on by poor storage procedures. Hence, the present study was conducted to assess the effect of various doses of gamma irradiation (0.25, 0.50, 0.75, and 1.0 kGy) on nutrient composition of dried mahua flowers stored at ambient temperature. Exposure of mahua flowers to irradiation improved the storability and preserved its nutritional value even after a year of storage. Nutrient composition of the flowers remained almost similar after 1 year storage with 0.25 kGy and 0.50 kGy doses of irradiation, while an increase in moisture and protein, decrease in ash and crude fiber content was observed in dried mahua flowers treated with gamma radiation doses of 0.75 and 1.0kGy. Among the bioactive compounds the phenol content was maintained at the same level with 0.25 kGy dose and the total flavonoid content was observed to be enhanced with all the four doses of irradiation. However, the antioxidant and carotenoid content of the flowers were found to be negatively affected by irradiation. The initial antioxidant content (1076.11 µg/100g) was reduced to 54.30 µg/100g after one year of storage. It can be inferred that modest doses of gamma irradiation (0.25 kGy and 0.50 kGy) might enhance the storability and retain quality attributes of dried mahua flowers and can be employed as an effective postharvest management approach for preserving and prolonging the shelf life of mahua flowers.

Keywords Mahua, Gamma irradiation, Nutrition, Bioactive compounds.

INTRODUCTION

The *Madhuca indica* tree, also known locally as the “Sweet Butter Tree” or “Mahua,” is one of the wonderful tree treasures found in Indian forests and is a member of the Sapotaceae family. Mahua is well known for its numerous applications, providing food security, and generating extra income for tribal

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members during the difficult summer time. The mahua tree is reported to have therapeutic benefits in all of its parts. The flowers are collected, sundried in open, and are preserved for later uses by the tribals. The dried flowers play a significant role in the indigenous people's diet and are used in many ways. Mahua flowers are used as a sweetening ingredient in several regional and traditional cuisines due to its high sugar content (sucrose, glucose, fructose, arabinose, and a little amount of maltose and rhamnose). Additionally, fermented flowers are used as raw material in preparation of traditional alcohol and alcoholic beverages. A sweet concoction made from boiled sun-dried flowers, tamarind, and sal seeds, is utilized as a rapid source of calories. Rice, ragi, or jowar together with mahua flowers and few root crops are used for preparing traditional cakes (Lakshmiprasanna and Aparna 2020). Kumari *et al*. (2018) reported that mahua flower is also utilized as a food ingredient for preparation of biscuit, cake, laddu, candy, bar, jam, jelly, sauces, and is a good source of nutrients like, protein, vitamins, carbohydrate, minerals, enzymes and organic acids.

The yearly productivity of mahua flowers in India is approximately 2 million tonnes (Singh *et al*. 2021). However due to hygroscopic nature, mahua flowers are highly perishable, accounting to 20–25 % of postharvest losses, leaving them viable only for use in distilleries and as animal feed (Behera *et al*. 2012). Because of this reason majority of the tribals are compelled to sell their collections right away, either with little or no value addition (Bakhara *et al*. 2016).

The only practice of post harvest management by tribals is sun drying of the collected mahua flowers followed by storage of the dried flowers for future uses. Grabowski *et al*. (2003) reported that over 20% of the world perishable crops are sun dried to increase shelf-life and promote food security. Open sun drying or shade drying is very simple method that does not require any capital investment, but the quality of product may be poor due to prolonged drying time and contamination during the process of drying, followed by further spoilage during storage of dried produce (Sagar and Kumar 2010).

Food irradiation is an economically viable technology for effective reduction of post harvest losses, extension of self-life of perishable commodity and improvement of hygienic quality of food (Labuza and Breene 1989). Joint Expert Committee on food irradiation (FAO/IAEA/WHO) in 1981 reported that medium dose of 1 to 10 kGy are necessary to extend the shelf life of dried fruits and vegetables (Chakraverty *et al*. 2003). Inayatullah *et al*. (1987) and Khan (1993) reported no significant changes due to irradiation in the composition, and no adverse effects were observed on the nutritional value or the sensory quality of dried fruits and nuts within a range of exposure doses. The objective of this study was to investigate the effect of various gamma irradiation doses on nutritional quality of sun dried mahua flowers.

MATERIALS AND METHODS

Freshly harvested and sundried flowers of mahua were collected from tribal area of Adilabad district of Telangana. Mahua flowers were sealed in perforated low-density polyethene ziplock covers and exposed to gamma rays at dose levels of 0.25, 0.5, 0.75 and 1.0 kGy in Co-60 gamma irradiation chamber (@ dose rate of 1.064 kGy/hr) at PJTS Agricultural University, Telanagana. The uniform dose delivery to the sample was ensured from the rotation of the chamber. Irradiated samples were stored at ambient temperature (25 to 28°C) for a period of one year. Nonirradiated sample served as control.

Nutrient composition: Moisture content of mahua flowers was determined by IS 1155:1968/4333(2):2002 method. Protein content was estimated as per AOAC 992.23. –Generic Combustion method, using Leco FP-528 Nitrogen Analyzer. Fat content was estimated as crude hexane extract of the dehydrated flowers using automatic Gerhardt Soxtherm extraction unit (AOAC 2003.06). Crude fiber content of the samples was determined by the procedure given by Association of Official Analytical Chemists (AOAC 962.09). Total ash was determined using IS 1155:1968 procedure. Energy and carbohydrate content was calculated by difference method (AOAC 2006). Total carotenoids and Beta carotene contents were estimated by AOAC (2000) methods.

Total phenol content: Using pyrocatechol as a reference phenolic compound, the total soluble phenolic compounds in mahua flower extract were quantified using the Folin-Ciocalteu reagent in accordance with the Slinkard method (Slinkard and Singleton 1997). 46 ml of distilled water was used to dilute 1 ml of the extract. Folin Ciocalteu reagent in an amount of one milliliter was then added, and the mixture was vigorously stirred. After adding 3 ml of Na_2CO_3 (2%) after 3 minutes, the mixture was left to stand for 2 hrs while being periodically shaken. Then, in a spectrophotometer, absorbance was measured at 760 nm against a blank made up of all the reaction ingredients minus the extract. According to an equation derived from standard pyrocatechol, the total phenol content of the extract was measured as micrograms of PE.

Total flavonoid content: The dowd technique was used to determine the total flavonoid content (Meda *et al*. 2005). Two ml of the extract solution was combined with two ml of methanol containing 2% aluminium trichloride ($AlCl_3$). The solution was let to rest for 10 minutes at room temperature, and then the absorbance at 415 nm was measured in a spectrophotometer in comparison to control samples. Using a method derived from a typical rutin graph, the total content of flavonoids in the extracts was calculated as micrograms of RE (rutin equivalent).

Total sugars and reducing sugars: Reducing sugars in the extracts were measured using the Somogyi and Nelson method (Nelson 1944, Somogyi 1945). Following the hydrolysis of the extract in HCl (1 N), total sugars were measured using the same technique.

TBARS (Thiobarbituric acid reactive substances) method (Nikols *et al.* 1994)

One gram of flower was homogenized in a motor and pestle with 10 ml of 0.1 M phosphate buffer (pH 7.8) and one percent of 0.05 M EDTA and centrifuged at 4,000 rpm for15 min at 5ºC. The clear supernatant extract was used for analysis. The reaction mixture contained 2.3 g of sample in phosphate buffer (0.26) ml, 0.1 M, pH 7.8), ferrous sulphate (0.05 mM), ascorbic acid (0.4 mM), potassium hydrogenphthalate 100 mM, pH 6.0) BHT (*Butylated hydroxytoluene*) (25 mM in 5 ml hexane) in a final volume of 2.4 ml. Contents of the tube were incubated for 30 min at 37ºC. TCA (Trichloro acetic acid) (0.75 ml,20 %) was added and centrifuged at 10,000 rpm for 30 min at 4ºC, followed by addition of TBA (Thiobarbituric acid) (0.5 % in 0.1 N NaOH). Distilled water was added to equalize the final volume to 3.24 ml. This was heated at 95ºC in a water bath for 30 min followed by immediate cooling in ice pack for 5 min. Finally, the reaction mixture was submitted to read the absorbance at 532 nm against TBA.

Statistical analysis

The results were subjected to statistical analysis with the window STAT program. Mean and standard deviation for three parallel replicates were calculated. Results were reported at 5 % level of significance.

RESULTS AND DISCUSSION

Untreated mahua flowers (control) spoiled within six months of storage when stored under ambient conditions due to heavy fungal infection. Hence, the control sample was discarded. However, irradiated mahua flowers could be stored upto one year. After one year of storage the proximate composition and other nutritional parameters were analyzed and are presented in Table 1. Irradiation was found to be efficient against a variety of pathogenic microbes by several other researchers as well (Pan *et al*. 2004 and Song *et al*. 2007).

Irradiation effect on proximate composition

The moisture content in the control sample was 16.88 ± 0.97 g/100g, which lead to fungal spoilage after storage of six months at ambient temperature. There was a significant ($p<0.05$) increase in moisture content (19.54 ± 0.20 to 23.01 ± 1.44 g/100g) on storage after 1 year in all the gamma irradiated samples, with no spoilage indicating the effectiveness of the radiation technology. However, among the radiation dosages, 0.25 and 0.50kGy doses had lower moisture accumulation among the four doses applied, while 0.75 and 1.0kGy dose had higher level of moisture. Nathawat *et al*. (2013) of Kachri (*Cucumis callosus)* which is not in agreement with our observations.

As per the results obtained, ash content was significantly ($p<0.05$) different in control and irradiated

Table 1. Gamma radiation effect on proximate composition of mahua flower.

Nutrient		Moisture (g/100g)	Ash (g/100g)	Protein (g/ 100g)	Fat (g/ 100g)	Crude fiber (g/ 100g)	CHO (g/ 100g)	Energy (Kcal/ 100g)
Before storage	Control	16.88 ± 0.97	2.43 ± 0.04	5.95 ± 0.01	0.62 ± 0.04	3.73 ± 0.22	74.14 ± 0.89	325.88 ± 3.91
After 1 year of storage	0.25kGy	19.54 ± 0.20	3.62 ± 0.13	5.46 ± 0.06	0.46 ± 0.01	3.78 ± 0.08	70.93 ± 0.37	309.64 ± 1.34
	0.50kGy	19.69 ± 0.05	3.74 ± 0.08	5.14 ± 0.19	0.47 ± 0.01	3.50 ± 0.43	70.98 ± 0.33	308.63 ± 0.50
	0.75kGy	22.51 ± 1.92	2.16 ± 0.03	6.26 ± 0.08	0.44 ± 0.02	3.18 ± 0.18	68.64 ± 1.83	303.50 ± 7.47
	1.0kGy	23.01 ± 1.44	2.28 ± 0.08	6.21 ± 0.25	0.39 ± 0.03	3.42 ± 0.58	68.12 ± 1.29	300.81 ± 5.94
CD		3.05*	0.21*	0.39*	0.06*	NA	2.90*	12.24*
SE(m)		0.82	0.06	0.11	0.02	0.25	0.78	3.29

* Significant @5% level of significance.

samples. However, it was observed that various radiation doses, did not affect the total ash content during the storage period. The protein content significantly ($p<0.05$) decreased during the storage period among the gamma radiated samples (0.25 and 0.50 kGy doses), when compared to control samples, while an increase in protein content was observed in 0.75 and 1.0kGy doses. Moderate doses of irradiation increase free amino acid content which might be the reason for increase in protein content at doses of 0.75 kGy and 1.0 kGy (Reeves 2015).

The crude fat content in control sample was significantly ($p<0.05$) higher while, there was no significant difference in the crude fat content among the irradiated samples except with the dose 1.0 kGy. Boonchoo *et al*. (2005) reported that within the period of storage (after 35 days) the free fatty acid content decreased continuously on irradiation dose of 6 kGy in brown rice. Al-Bachir (2004) reported no significant difference in fat content between irradiated and non-irradiated samples of walnuts. However, in the previous study mentioned, samples were stored from 3 months to 6 months, while the present study stored the samples for 1 year, which could be the reason for differences in our observations. Crude fiber content was not affected in irradiated mahua flowers, during the storage. Similar observations in crude fiber content with irradiation was also reported by (Bahraini *et al*. 2017). There was a significant ($p<0.05$) decrease in both carbohydrate content and energy content in control and irradiated samples. Similar results were reported by Nathawat *et al*. (2013), who reported progressive decrease in starch content and enhanced breakdown of starch during storage as the irradiation dose was raised.

Irradiation effect on bioactive compounds

A significant effect of irradiation on bioactive compounds was observed in the study (Table 2). Decrease in the total and β-carotene content was observed with the increase in the irradiation dose and upon storage. Total carotene content of 13.10 µg/100g in non irradiated mahua flowers reduced to 1.65 µg/100g as the dose of radiation was increased to 1.0 kGy. A similar decreasing trend was observed in the β-carotene content also (6.92 µg/100g in control reduced to 0.80µg/100g with increasing radiation dose of 1.0 kGy). These results are consistent with the report of Kilcast (1994). A significant decrease in the antioxidant content was also observed with irradiation doses (from 1076.11 to 54.30 µg/100g). This decrease in antioxidant content can be correlated with reactive oxygen species neutralization, which are produced by irradiation (Sajilata and Singhal 2006). There was a reduction in total phenolic content with the increase in dose of irradiation. However, phenolic content in control and 0.25 kGy treated mahua flowers was similar and maintained till the end of one year storage.

Table 2. Gamma radiation effect on bioactive compounds of mahua flower.

Nutrient		Total carotene (µg/100g)	β carotene (µg/100g)	Total aox activity (µg/100g)	Total phenols (mg GAE/100g)	Total flavonoids (mg QE /100g)
Before storage	Control	13.10 ± 1.56	6.92 ± 0.06	1076.11 ± 5.50	225.83 ± 1.06	560.45 ± 3.46
After 1 year of storage	0.25kGy	6.34 ± 1.90	1.76 ± 0.57	54.30 ± 0.71	224.61 ± 0.67	936.00 ± 1.83
	0.50kGy	3.33 ± 0.04	1.16 ± 0.06	57.75 ± 3.46	215.37 ± 1.62	945.00 ± 1.07
	0.75kGy	2.91 ± 0.45	1.04 ± 0.00	55.41 ± 0.86	139.23 ± 1.21	654.00 ± 1.62
	1.0kGy	1.65 ± 0.02	0.80 ± 0.02	56.54 ± 3.82	149.70 ± 1.27	628.00 ± 1.49
CD		2.94*	0.68*	8.96*	12.69*	10.49*
SE(m)		0.79	0.18	2.41	3.42	2.82

* Significant @5% level of significance.

These results signified the sensitivity of phenolic compounds to the ionizing radiation (Carocho *et al.* 2012). A significant increase in the flavonoid content was observed upon irradiation with 0.25 and 0.50kGy doses, while a mild increase was observed with 0.75 and 1.0kGy gamma radiation doses. Taheri *et al.* (2014) also reported that acute gamma irradiation increases the amount of certain polyphenols and flavonoids in the leaves of *Curcuma alismatifolia* plants whose rhizomes have already undergone radiation treatment. Interesting result observed in this study was that, though there was an increase in the flavonoids content which contribute to the antioxidant activity, reduction in antioxidant activity was observed after irradiation.

The total sugar content of 42.02 g/100 g in the non irradiated mahua flowers did not change significantly with the gamma radiation doses of 0.5, 0.75 and 1 kGy. However, a slight reduction in reducing sugar content was observed with irradiation (Fig. 1). Khan *et al.* (2018) reported no significant effect of irradiation on total sugar content. Results reported in the literature on the effect of irradiation on sugar content are ambiguous in various food matrices. Auda *et al.* (1977) found that irradiation had no effect on reducing sugar and major components, whereas Al-Kahtani *et al.* (1998) showed that irradiation at doses ranging from 0.3 to 0.9 kGy followed by 3 or 6 months storage at room temperature, sugar contents increased gradually with increasing storage time. Roushdi *et al.* (1981) observed that irradiation of dried corn increased the reducing sugars and total soluble sugars in proportion to dose.

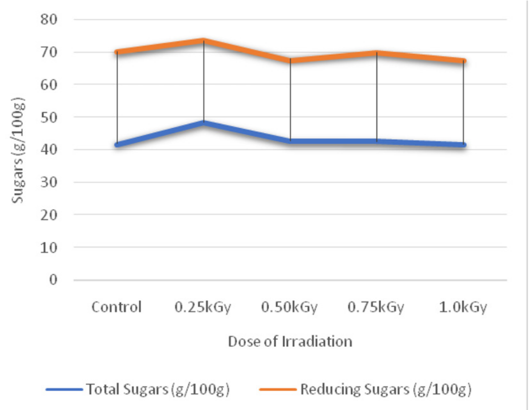

Fig. 1. Effect of irradiation on total and reducing sugar content.

CONCLUSION

Gamma irradiation at 0.25 and 0.50kGy was found to be very effective and sufficient in extending the shelf life of dried mahua flowers, with good retention of protein and crude fiber content. However, carbohydrates and energy content reduced significantly during storage. There was a decrease in the bioactive components like total carotene, β carotene and total antioxidant activity on increasing doses of gamma radiation from 0.25kGy to 1.0kGy. The total phenols content remained constant with 0.25kGy to 1.0kGy doses, while total flavoring content improved with the same doses. It is concluded that gamma irradiation of dried mahua flowers with 0.25kGy and 0.50kGy improves shelf life upto one year, while retaining good amount of nutrients during storage.

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