Induction of gamma irradiation for microorganism decontamination of dried lotus pollen (*Nelumbo nucifera*)

S Sajjabut¹, W Pewlong¹, J Eamsiri¹, S Chookaew¹, K Kemtong¹ and L Maikaeo¹

¹Thailand Institute of Nuclear Technology (Public Organization) 9/9 Moo7 Saimoon, Ongkharak, Nakhon Nayok 26120, Thailand

E-mail: saksajja@yahoo.com

Abstract. Gamma irradiation has been known for its great effect for inactivating microorganisms in various foods and it has been a safe method for food decontamination. In this present study, the dried lotus pollen was conducted to determine the effect of using gamma irradiation on the microbial load and antioxidant properties. The dried pollen lotus samples were irradiated with gamma irradiation at doses of 5 and 10 kGy and the total plate count (TPC) and total yeast and mold (Y&M) were determined. The study revealed that high microbial level of TPC and Y&M were found in non-irradiated. At dose of 5 kGy showed that gamma radiation was able to reduce the microorganism contamination in dried lotus pollen. The result permitted to accomplish the satisfactory microbial content for Thai community product standard of dried herb number 480/2547. In part of the antioxidant property, there was no any significant difference among the non-irradiated and irradiated samples in 60% ethanol extract. Surprisingly, total phenolic content and DPPH were increased by gamma radiation in hot water extract. Therefore gamma irradiation at 5 kGy could be a potential method for microbial decontamination of dried pollen lotus to improve hygienic quality.

1. Introduction

Sacred lotus or East Indian lotus (*Nelumbo nucifera*) is a large water plant. The pollen of lotus has been known for its medical potential. There are several pharmacological benefits such as to decrease blood sugar, to nourish the heart and to eliminate the toxic substance. The various bioactive substances have been found in the pollen, especially flavonoids such as quercetin, luteolin, isoquercitrin and luteolin-glucoside [1]. These substances have been known that there are properties to benefit health [2]. The pollen has several therapeutic advantages to the heart, spleen, and liver. In addition, many traditional medicines have lotus pollen as a necessary ingredient in their formularies [3]. Recently, lotus pollen is produced for health supplement products, especially the pollen tea. Lotus pollen showed high content of antioxidant activity when extracted with hot water [4].

Gamma irradiation has been accepted as a method to decontaminate the pathogenic microorganisms on herb and food. Food irradiation is an important technique for the treatment of foods that may be contaminated with pathogenic microorganisms. If utilized suitably, irradiation can be an effective path to decrease the incidence of foodborne disease and to treat a variety of potential problems in food. The present work was mainly undertaken to investigate the effect of gamma irradiation on the microbial elimination and the antioxidant properties alteration in dried lotus pollen.
2. Materials and Methods

2.1. Gamma irradiation and sample preparation
The dried lotus pollen was purchased from Phra Nakhon Si Ayutthaya province in Thailand. The dried pollen was packed into the polyethylene zip bag (5 g / bag). The bags of pollen were irradiated in room temperature at doses 5.0 and 10.0 kGy with a dose rate of 3.2 kGy/h using gamma irradiation facility (Gamma chamber 5000) at Thailand Institute of Nuclear Technology (Public Organization) (TINT). The irradiated and non-irradiated samples (each sample as 1.0 g) were extracted with 60% ethanol (100 mL) for 1 h at room temperature in an ultrasonic bath and extracted with hot water (90 °C) by standing them for 10 minutes. The sample suspensions were centrifuged and the supernatants were filtered through a filter paper No.4. The filtrates were stored at –20°C until the analysis.

2.2. Microbial analysis
The microbial study was conducted to determine the total viable bacterial count and total yeast and mold count (CFU/g) in the irradiated and non-irradiated dried lotus pollen by the method of FDA-BAM[5-6].

2.3. Chemical qualities evaluation

2.3.1 Determination of total phenolic content. The filtrate of samples from 2.1 were also used in this procedure. The total phenolic content was estimated using the Folin-Ciocalteu assay according to the method developed by Velioglu et al.[7]. First, 0.75 mL of 10-fold diluted Folin-Ciocalteu reagent and 100 μL of the methanolic extract were placed in a test tube. The mixture was mixed and allowed to stand at room temperature for 5 min. Then, 0.75 mL of 6% (w/v) sodium carbonate solution was added. The mixture was homogenized and allowed to stand at room temperature for 90 min. The total phenolic content was determined via the absorbance measurements at 725 nm using a spectrophotometer. The standard calibration curve was plotted using gallic acid at the concentrations of 0.02 - 0.1 mg/mL. The total phenolic content was expressed in mg gallic acid equivalent (GAE)/g sample.

2.3.2 Determination of free radical scavenging power (DPPH assay). Determination of free radical scavenging power was performed as previously described by Khattak et al. [8] with slight modifications. First, 100 μL of each extract was added to 900 μL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol solution (150 μM) and the solution was shaken vigorously. After incubation at room temperature for 15 min in darkness, the absorbance of each solution was determined at 517 nm. The free radical scavenging power was expressed as ascorbic acid equivalent (AAE)/g sample.

2.4. Statistical analysis
All measurements were done in triplicate. Results were expressed as mean ± standard deviation (SD). Data were analysed using one way ANOVA and DUNCAN test (SPSS software version 21) for differences between the control and the irradiated samples for all parameters.

3. Results and discussion

3.1 Microbial decontamination
The principal objective of this study was the elimination on the microorganism in dried lotus pollen by gamma irradiation. The result of the effect of gamma irradiation on the microbiological quality was shown in Table 1. The initial populations of the total viable bacterial and total yeast and mold counts of the lotus pollen samples were observed. There were about 1.9 x 10^5 CFU/g for total aerobic counts and 2.3 x 10^3 CFU/g for total yeast and mold counts. The amount of initial microbial load was decreased after gamma irradiation. At dose of 5 kGy, total viable bacterial and total yeast and mold counts were 5.5 x 10^3 and 13 CFU/g, respectively. At this dose, gamma irradiation can reduce contamination by 2 log cycles from the initial microbial load. The result allowed to achieve the acceptable microbial level.
for Thai community product standard of dried herb number 480/2547 (total viable bacterial count \(\leq 5 \times 10^5 \text{ CFU/g}\), total yeast and mold count \(\leq 100 \text{ CFU/g}\)). Furthermore, 10 kGy of gamma irradiation can decrease the initial microbial population to be almost absent. Thus, gamma irradiation at 5 kGy was sufficient to eliminate contaminated bacteria, yeast and molds. The results of this study were similar to the report of Pewlong et al.\[9\], Fanaro et al.\[10-11\] and Phianphak et al.\[12\]. The report of Pewlong et al. showed that the microbial population on dried ginger was reduced by 3 log cycles from the initial load by gamma irradiation at dose 5 kGy. Similar to Fanaro et al., they studied the effect of gamma irradiation on the leaves of green tea and black tea, finding no fungal growth on 5 kGy irradiated leaves of either variety. Seventeen species of Thai herbs were studied for contamination by using gamma irradiation doses of 7.7 and 8.8 kGy by Phianphak et al. It was found that no microorganisms remained after gamma treatment and the color, aroma and texture of the herbs did not change.

Table 1. Effect of gamma irradiation on the total viable bacterial and total yeast and mold counts of dried lotus pollen.

| Dose (kGy) | total viable bacterial counts (CFU/g) | total yeast and mold counts (CFU/g) |
|------------|-------------------------------------|-----------------------------------|
| Non-irradiated | 1.9 \times 10^5 | 2.3 \times 10^3 |
| 5.0 | 5.5 \times 10^3 | 13 |
| 10.0 | 60 | < 10 |

CFU = Colony Forming Unit

3.2 Total phenolic content and DPPH radical scavenging activity

The effects of gamma irradiation at 5.0 and 10.0 kGy on total phenolic content and DPPH value in dried lotus pollen presented in Table 2. In the present study, a significant increase (\(p < 0.05\)) in total phenolic content was observed on the irradiated in comparison with non-irradiated lotus pollen in the hot water extract. The results showed that the irradiated samples of 5.0 and 10.0 kGy had a total phenolic content around 30% higher than the non-irradiated sample, while irradiated pollen at 10.0 kGy had the highest value. However, no significant difference was found in 60% ethanol extract between irradiated and non-irradiated pollen. The literature relating to the effect of gamma radiation on a total phenolic content of dried lotus pollen has not been reported. However, there have been a few reports for other plant materials for increasing in a total phenolic content due to a rising the gamma irradiation dose. For example, Zhu et al.\[13\] reported that total phenolic content in the irradiated black rice at 8 kGy was higher than in the non-irradiated sample. Harrison and Were\[14\] also found an increase in total phenolic content of irradiated almond skin extract as compared to non-irradiated. Moreover, Khattak et al.\[8\] indicated that the phenolic content of \textit{Nigella staiva} seed was increased by gamma irradiation at dose of 16 kGy. These increases in total phenolic contents were related to the degradation of tannins \[15\] and changed in the conformation of the molecules \[16\] from the effect of the irradiation treatment. In present study, 60% ethanol and hot water were used as solvent to extract the samples. Generally, 60 % ethanol is a solvent, and has been known for its ability to extract bioactive substances from dried herbs or plants for antioxidant properties analysis. Certainly, 60% ethanol will show total phenolic content and DPPH value higher than when extracted in hot water. However, hot water was utilized in this study as it is an agent that normally used in everyday life, drinking tea. This study showed that the irradiated samples of 5.0 and 10.0 kGy had a total phenolic content higher than the non-irradiated sample in hot water extract.

From this result, we suggest that the water-soluble substances may be released from the structure of plant tissue due to gamma irradiation. These released substances will be further researched in the future. In contrast, Koseki et al.\[17\] have reported that total phenolic content in dried rosemary was diminished by gamma irradiation at doses 10-30 kGy. The difference in the effect of irradiation on total phenolic content may be due to plant type, the state of sample (wet, solid, paste or dry) phenolic content composition, extraction solvent, temperature and dose of gamma irradiation \[8\].
Table 2. Effect of gamma irradiation on total phenolic content and DPPH in 60% ethanol and hot water extract of dried lotus pollen.

| Dose (kGy) | Total phenolic content (mgGAE/g) | DPPH assay (mgAAE/g) |
|------------|----------------------------------|----------------------|
|            | 60% ethanol                      | Hot water            | 60% ethanol | Hot water |
| Non-irradiated | 29.25 ± 1.94^a                   | 7.40 ± 0.34^a       | 24.42 ± 0.13^a | 9.72 ± 1.70^a |
| 5.0        | 30.33 ± 0.38^a                   | 10.09 ± 0.67^b      | 24.42 ± 0.04^a | 11.79 ± 1.48^ab |
| 10.0       | 28.24 ± 0.61^a                   | 11.98 ± 1.07^c      | 24.35 ± 0.49^a | 14.20 ± 1.00^b |

Superscripts with different letters within same column indicate significant differences at $p < 0.05$ within the same column.

GAE = Gallic Acid Equivalent
AAE = Ascorbic Acid Equivalent

DPPH assay has been one of the most widely used methods for screening the antioxidant activity of plant or herbal extracts. The assay is based on the measurements of the antioxidants ability to scavenge the stable radical DPPH. Table 2. represented the DPPH radical scavenging activity of irradiated and non-irradiated dried lotus pollen that expressed in terms of ascorbic acid equivalents. This study showed that DPPH value in the hot water extracts of the irradiated pollen was also higher than the non-irradiated pollen. The DPPH value in the hot water extracts of the non-irradiated sample was 9.72 ± 1.70 mgAAE/g and the irradiated samples at doses of 5.0 and 10.0 kGy were 11.79 ± 1.48 and 14.20 ± 1.00 mgAAE/g, respectively. The similar effect of gamma irradiation on scavenging activity of DPPH radical has been studied in other products. Rajurkar et al. [18] also reported an increase in DPPH radical scavenging activity of gamma irradiated Justicia adhatoda. Perez et al. [19] reported that DPPH radical scavenging activity of rosemary leaf extracts was improved by irradiation. Hussain et al. [20] showed that DPPH radical scavenging activity in irradiated fenugreek and spinach were higher than non-irradiated sample. The present study reported that DPPH value in the hot water extracts of dried lotus pollen was increased by irradiation. This study showed similar result of total phenolic content increase in hot water extract of irradiated lotus pollen. Polyphenolic compound has been well known as a great source of antioxidant compounds. Therefore, it can be seen that the increase of total phenolic content will result in rising the DPPH value. On the other hand, the DPPH value in 60% ethanol extracts of dried lotus pollen was not affected by gamma irradiation.

4. Conclusion

The results from this study demonstrated that gamma irradiation at doses of 5 – 10 kGy could significantly reduce the levels of total viable bacterial and total yeast and mold counts in dried lotus pollen. This study showed that the amount of microorganisms can be reduced according to Thai community product standard of dried herb by gamma radiation at dose 5 kGy. The present study showed similarity in terms of total phenolic content and DPPH in irradiated and non-irradiated pollen of 60% ethanol extract. Surprisingly, gamma radiation could increase the total phenolic content and DPPH value in hot water extract. This research concluded that gamma irradiation at 5 kGy could be a potential method for microbial load decontamination of dried pollen lotus to improve hygienic quality. Not only gamma irradiation can be considered effective in decreasing microbial load but it can also increase the antioxidant property in water extract of dried lotus pollen.

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