Effects of gamma irradiation on antioxidant properties and microbial contamination in *Boletus griseipureus* Coner

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**Abstract.** The aim of this research is to investigate effect of gamma irradiation on antioxidant properties and microbial contaminants in *Boletus griseipureus*. Samples were ground into powder and later subject to gamma irradiation at 2.5, 5, 7.5 and 10 kGy. The non-irradiated and irradiated samples were then analyzed for their antioxidant properties by techniques including 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), total phenolic content, beta glucan content and total triterpenoids. In addition, the microbial contamination was also evaluated. In terms of antioxidant properties, results showed that gamma irradiation did not result in significant effects on FRAP, total phenolic content, beta glucan content, and total triterpenoids. However, at 10 kGy of gamma irradiation, DPPH radical scavenging activity notably increased (p≤0.05). In term of microbial contamination, irradiation dose of 5 kGy reduced the microorganism to meet standard level and irradiation dose of 10 kGy was found to decrease microorganism to complete absence. Therefore, this research suggested that a minimum irradiation dose of 5 kGy can serve as a useful treatment for preserve its quality and prevent microbial contamination.

1. Introduction

In Thailand there is a great diversity of wild edible mushroom species such as *Amanita princeps*, *Russula luteotacta*, *Tricolina crissum*, *Lentinus edode* [1] and *Phlebopus portentosus* [2]. Wild edible mushrooms are an important natural resource with progressively higher market demand because they are generally low in energy and fat but high in protein, fiber and carbohydrate [3].

*Boletus griseipureus* was wild ectomycorrhizal edible mushroom. It grows in symbiotic relationship with *Melaleuca leucadendron* (SaMed Kho or cajeput tree) [4] and *Acacia mangium* [5] in forest soil once in a year during rainy season. The morphological characteristic of *B. griseipureus* (Figure 1) has an average of pileus diameter 3.0 – 6.0 cm with convex to plane in shape. The pileus range in color from grey to purple or light purple with the surface covered with short, soft hairs in grey to black color when young. The tube part is small and firm with pallid white color when young before the color later matured to pinkish or pale brown. The shape of stipe, which is purple in color, is cylindrical to clavate with an average diameter and length of 1.5 – 3.0 cm and 3.0 – 6.0 cm, respectively. The size of basidia is 24.0 – 40.0 x 8.0 – 10.5 micron with clavate shape. The average size of basidiospores is 3.1 – 4.9 x 7.8 – 10.7 micron. The basidiospores can be seen in smooth, cylindric shape with pinkish to pale brown in color [6]. Some reports have been published on its nutritive values, anticancer [5], antioxidant, and antimicrobial activities [6, 7].
B. griseipureus was grown on forest soil. After harvest, it causes a reduction in the vegetative cells of microorganisms, which gives rise to a flora of bacteria and fungi that have the ability to survive for a long period. However, a serious problem with its microbial contamination can affect the safety and health of the consumers. Recently, the main requirement focuses on residual microbial contamination. The conventional methods of decontamination were fumigation with gaseous ethylene oxide or methyl bromide, which are now prohibited or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons [8]. Gamma irradiation has shown to be an effective method used for decontamination of microorganisms with the advantage of high penetration to treat products that are already stored in packages. The Joint Expert Committee on Food Irradiation (JECFI) convened by Food and Agriculture Organization (FAO), World Health Organization (WHO) and International Atomic Energy Agency (IAEA) have concluded that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard and requires no further testing [9].

2. Materials and Methods

2.1. Sample preparation and irradiation
Fresh B. griseipureus were purchased from the inhabitant people who collect from forest areas of Rattaphum district within Songkhla Province, Thailand. The samples were dried with hot air dry at 55 °C and ground to powder. After that it was packed in aluminium foil bags. Samples were irradiated by gamma rays with a fixed dose rate of 2.62 kGy/hr for a total dose of 0, 2.5, 7.5 and 10 kGy. Irradiation at each dose was done in triplicate.

The 100 mg samples of each irradiation dose were soaked with 10 ml of water and methanol before subjected to extraction by an ultrasonic cleaner for 60 min. The mixture was centrifuged at 8000 rpm at 25 °C for 5 minutes, and then its supernatant was collected to be used as sample solution and stored at 4 °C until required for analysis. The mushroom water extraction was prepared for the determination of total phenolic content, DPPH free radical scavenging activity, ferric reducing antioxidant power and beta glucan content. In a part of total triterpenoid using the methanolic extract.

2.2. Antioxidant properties

2.2.1. DPPH free radical scavenging activity. The DPPH free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical with slight modification. In brief, a 0.2 mM solution of DPPH radical solution in methanol was prepared. Sample solutions of 100 µl each was added to 900 µl of DPPH radical solution and incubated at ambient temperature for 15 min in the dark. The absorbance of each solution was measured spectrometrically at 517 nm. The ascorbic acid was used as a reference compound and the free radical scavenging activity was expressed as the ascorbic acid equivalent (AAE) per gram of sample [10].
2.2.2. Ferric Reducing Antioxidant Power (FRAP). The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM FeCl\textsubscript{3}.6H\textsubscript{2}O in the proportion of 10:1:1. Freshly prepared working FRAP reagent was pipetted using 2700 µl mixed with 300 µl of the sample solutions. Incubate the mixed solutions for 30 min avoiding light at ambient temperature. The absorbance at 615 nm was recorded against a reagent blank. The calibration curve was prepared by plotting the absorbance at 615 nm versus different concentrations of FeSO\textsubscript{4}. The FRAP values were expressed as the FeSO\textsubscript{4} equivalent in µmol per gram of sample [11].

2.2.3. Total phenolic content. The total phenolic content was estimated by Folin-CiTheocalteu assay according to a slightly modified method. 100 µl of sample solution was mixed with 750 µL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 25 °C for 5 min; 750 µL of 6% sodium bicarbonate solution was added to the mixture. After 90 min at 25 °C, absorbance was measured at 725 nm. Results are expressed as gallic acid equivalents (GAE) in mg per gram of sample [12].

2.2.4. Beta glucan content. The Beta-D-glucan was determined using an enzymatic yeast beta-glucan assay kit (Megazyme International Ireland Ltd.) according to the manufacturer’s instruction [13].

2.2.5. Total triterpenoids. The content of total triterpenoids was determined with a slight modification. Briefly, after a 500 µL sample solutions were dried in a water-bath, 1 mL new mixed of 5% (W/V) vanillin-acetic solution and 1.8 mL sulfuric acid were added, mixed and incubated at 70°C for 30 min. Then the mixed solution was cooled with water for 5 min and added with 5 ml acetic acid. The absorbance was measured at 550 nm against blank using a spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The total triterpenoids content is expressed as milligram ursolic acid equivalent/gram dry weight [14].

2.3. Microbial contamination
About 2 g of each samples were dissolved in 18 ml of 0.1 % normal saline. After that, each sample was analyzed for microbiological quality such as total viable bacteria counts (TVC) and total yeast and mold counts (TYM). Microbiological assays were performed using the AOAC standard protocol [15].

2.4. Statistical analysis
All determinations were obtained from triplicate measurements and results were expressed as mean ± standard deviation. Statistical analyses were performed with one-way ANOVA and Duncan’s test was applied for comparison of the mean values. p≤0.05 was regarded as significant. All statistical analyses were performed using SPSS software (version 16).

3. Results and discussion

3.1. Antioxidant properties

3.1.1. DPPH free radical scavenging activity. DPPH (2, 2-diphenyl-1-picrylhydrazy) is used to evaluate the ability of an antioxidant based on the extent of protons being donated that can be detected from the change in color from deep purple to yellow [16]. The reduction of DPPH by antioxidants in the \textit{B. griseipureus} water extracts expressed as ascorbic acid equivalent (AAE) per gram. DPPH activity exhibited ranges from to 3.02±0.23 to 3.49±0.16 mgAAE/g (Table 1). These results, based on water extracts, showed higher DPPH values compared to those obtained from methanol and ethanol extracts (0.20±4.4 and 0.08±0.4 mg vitamin c/g sample) [7]. However, the irradiated samples at 2.5-7.5 kGy were found to be no significant (p>0.05) when compared to the non-irradiated samples. At 10 kGy radiation dose, the DPPH activity was significantly increased (p≤0.05). The results were also
comparable to other wild edible mushroom such as *Arenaria montana* after 10 kGy irradiated. The methanolic extracts showed higher DPPH activity than the non-irradiated sample [17].

**Table 1.** Effects of gamma irradiation on DPPH activity, FRAP values and total phenolic content of *Boletus griseipureus* Corner mushroom.

| Dose (kGy) | DPPH (mgAAE/g) | FRAP (µmolFeSO₄/g) | Total phenolic content (mgGAE/g) |
|------------|----------------|---------------------|--------------------------------|
| 0          | 3.13±0.14ᵃ     | 32.26±1.61ᵃ         | 5.58±0.50ᵃ                      |
| 2.5        | 3.02±0.23ᵃ     | 31.66±2.83ᵃ         | 5.27±0.29ᵃ                      |
| 5.0        | 3.21±0.31ᵃ     | 34.16±1.16ᵃ         | 5.88±0.30ᵃ                      |
| 7.5        | 3.14±0.12ᵃ     | 32.24±0.27ᵃ         | 5.55±0.22ᵃ                      |
| 10.0       | 3.49±0.16ᵇ     | 36.68±0.86ᵃ         | 5.86±0.16ᵃ                      |

Each value is expressed as mean±standard deviation (n=3). Means with same letters within a column are non-significantly different (p≥0.05).

GAE = Gallic Acid Equivalent
AAE = Ascorbic Acid Equivalent

3.1.2. **Ferric Reducing Antioxidant Power (FRAP) and total phenolic content.** The FRAP value was determined from the change in absorbance at 596 nm owing to the formation of a blue colored Fe²⁺ compound from colorless, oxidized Fe³⁺ by the action of electron donating antioxidants [11] and the increasing absorbance suggests an increase in reducing power [18]. The FRAP values of both non-irradiated and irradiated samples (Table 1) were shown to be within the same range, 31.66±2.83 to 36.68±0.86 µmolFeSO₄ /g (p≤0.05).

The total phenolic content (TPC) of *B. griseipureus* extracts was carried out using the standard curve of gallic acid and presented as gallic acid equivalents (GAE) per gram. The phenolic content of both non-irradiated and irradiated samples (Table 1) were shown to be within the same range, 5.27±0.29 to 5.88±0.30 mgGAE/g.

Though various researchers have worked on effects of gamma irradiation on antioxidant properties, there is not yet a report on *B. griseipureus*. However, earlier research studies showed different results for the effect of gamma irradiation on the antioxidant properties of wild edible mushroom. Similar results were reported on *Agaricus bisporus* that had been irradiated at doses of 1, 3, and 5 kGy in which the total phenolic content and antioxidant capacity were not influenced by irradiation [19].

**Table 2.** Effects of gamma irradiation on beta glucan, and total triterpenoids of *Boletus griseipureus* Corner mushroom.

| Dose (kGy) | Beta glucan (%w/w) | Total triterpenoids (mgUAE/g) |
|------------|---------------------|-------------------------------|
| 0          | 13.45±1.44ᵃ         | 12.72±0.12ᵃ                   |
| 2.5        | 13.20±1.34ᵃ         | 13.67±0.95ᵃ                   |
| 5.0        | 13.11±0.25ᵃ         | 13.00±0.84ᵃ                   |
| 7.5        | 13.51±1.38ᵃ         | 11.58±0.64ᵃ                   |
| 10.0       | 13.98±0.19ᵃ         | 12.15±1.01ᵃ                   |

Each value is expressed as mean±standard deviation (n=3). Means with same letters within a column are non-significantly different (p≥0.05).

3.1.3. **β-glucan content.** β-glucan is one of the polysaccharides that has shown a number of health benefits [20]. Mushroom β-glucans are known as biological response modifier which are used for the treatment of various cancers as well as possess the immune modulatory activity [21]. The *B. griseipureus* chosen in this study provides a rich source of fungal β-glucan. The β-glucan content of non-irradiated was 13.45±1.44 (%w/w) which was similar to the values obtained from irradiated
samples, 13.20±1.34 to 13.98±0.19 (%w/w) (Table 2). The results are consistent with Hericium erinaceus in previous studies [22].

3.1.4. Total triterpenoids. Among the large number of terpenes, triterpenoids are exclusively found in certain macrofungi, mainly Basidiomycetes, and are recognized for their biological activities and medicinal purposes [23]. In this study we reported the extraction of total triterpenoids isolated from B. griseipuixreus. The results were 12.15±1.01 to 13.67±0.95 mgUAE/g, which did not significantly change as the irradiation doses increased from 2.5 to 10 kGy.

3.2. Microbial contamination
Total elimination of the microorganism is the principal aim of irradiation. The effects of gamma irradiation at various doses on total viable bacterial counts (TVC) and total yeast and mold counts (TYM) are shown in Figure 2. The results of TVC indicated that the non-irradiated samples of B. griseipuixreus were contaminated with bacteria at the level of 6.25x10⁴ cfu/g. The values exceeded the level of 1.0x10⁶ cfu/g reported by WHO as the maximum permissible total count level. The initial TYM of non-irradiated samples were 7.6x10¹ cfu/g, whereas that of samples irradiated at 5 kGy were 2x10¹ cfu/g. TYM were eradicated after irradiation at 5 kGy. The irradiation at 5 kGy was sufficient to eliminate the total bacteria and total yeast and mold, while irradiation at 10 kGy resulted in a complete absence of microorganisms. Both microbiological analyses decreased linearly with absorbed doses. These results were in good agreement with previous study on Agaricus bisporus. They found that gamma irradiation doses 1-3 kGy led to decreased microbial contamination [24, 25, 26].

![Figure 2. Effect of irradiation on total viable bacterial counts (TVC) and total yeast and mold counts (TYM) of Boletus griseipuixreus.](image)

4. Conclusion
The effects of different gamma irradiation doses were assessed by measuring the changes in microbial contamination and antioxidant properties from wild edible mushroom Boletus griseipuixreus. The estimated profiles of antioxidant properties were shown to be similar non-irradiated and irradiated samples. In term of microbial contamination, gamma irradiated dose of 5 kGy could reduce microbial contamination to standard level. Therefore, the use of gamma radiation could improve the hygienic quality and preserve antioxidant properties of dried mushroom.
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