

Effects of Gamma Irradiation on Microbial Load and Chemical Properties for Preserve Dried Shiitake Mushroom Powder

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ABSTRACT

Mushrooms are easily susceptible to microorganism spoilage and insect damage. Thus, the process of preservation is necessary for extending mushroom shelf-life. The objective of this study was to investigate the effects of gamma radiation on microbial analysis, antioxidant activities and chemical constituents of dried shiitake mushrooms. The shiitake powder was irradiated with gamma radiation at doses of 2.5, 5.0, 7.5 and 10 kGy. The irradiated samples were extracted with distilled water using ultrasonic bath. The extract was examined for microbial load, antioxidant activities by means of DPPH, FRAP and total phenolic content. Chemical properties were analyzed for total protein content and β -D-glucan content. The result showed that the total bacterial count was eliminated at a dose of 5 kGy and 2.5 kGy for yeast and mold, respectively. Pertaining to antioxidant activities; Increasing gamma irradiation dose, decreasing DPPH, FRAP and total phenolic content of shiitake powder were observed. At the dose of 10 kGy, the lowest DPPH value 2.07 ± 0.02 mgAAE/g, was achieved. The non-irradiated sample showed the highest value (3.16 ± 0.11 mgAAE/g). The FRAP values of irradiated samples ranged from 40.24 ± 0.41 to 3.16 ± 0.11 $\mu\text{molFeSO}_4/\text{g}$. At the dose of 2.5, 5.0, 7.5 and 10 kGy significantly reduced total phenolic acid content (9.00 ± 0.09 , 9.05 ± 0.47 , 8.12 ± 0.48 and 8.15 ± 0.10 mgGAE/g), respectively as compared with control (9.66 ± 0.13 mgGAE/g). Irradiation at the dose of 10 kGy did not significantly affect to total protein content and β -D-glucan content. The total protein contents

ranged from 28.45 ± 0.24 to 28.60 ± 0.07 %w/w. The amount of β -D-glucan in non-irradiated and irradiated samples varied from 23.27 ± 1.21 to 25.11 ± 0.51 %w/w. The results of this investigation suggested that gamma irradiation could be applied to preserve the dried shiitake mushroom powder for food seasoning.

Keywords: Shiitake, Gamma irradiation, *Lentinus edodes*

INTRODUCTION

The Shiitake (*L. edodes*) mushroom belongs to the Omphalotaceae family. It is an edible medicinal mushroom that is traditionally consumed in East Asian countries. In Thailand, *L. edodes* is commonly known as Hed Hom. It is well known for its delicious taste and its nutritional values. Shiitake mushrooms also contain several bioactive compounds such as polysaccharides (β -D-glucan), ergosterol, vitamin B₁, B₂, C and minerals (Mattila et al., 2001). The antioxidant activity of shiitake mushroom extracts was found to be correlated with their polysaccharide and total phenolic content (Cheung et al., 2003). The mushroom water extracts contained various polysaccharides, phenolic compounds, and small proteins such as lectins, each of which may have its own biological effects (Kozarski et al., 2012). The shiitake mushrooms are easily perishable and tend to lose their qualities 2-3 days after harvest. The high respiration rate and a tendency to turn brown (enzymatic activity), and susceptibility to lose water result in quick deterioration (Simon et al., 2005; Jiang et al., 2010). Therefore, preservation methods are to extend the shelf-life of the mushroom. Freezing and drying techniques are the most popular methods, especially sun-drying and hot air oven. (Ma et al., 2013).

Irradiation is known to be a safe technology for treating food products using ionizing radiation. Gamma ray ionizing radiation is used to extend the shelf-life of food products and results in the inactivation of foodborne pathogens (Jiang et al., 2010). Research on disinfection irradiation, spores inhibition and extension of shelf-life has been undertaken by many (WHO, 1981; Farkas, 1998).

Irradiation at an average dose of 10 kGy has shown no toxicological hazards and no specific microbial and nutritional problems (WHO, 1981). Gamma irradiation at a dose 0.25–1.0 kGy is recommended for insect disinfestation and at 6–10 kGy to control mold growth (Diehl, 1995). Radiation doses in the range of 2.5–10.0 kGy were selected for this study. The purpose of this study was to determine the effects of gamma irradiation on microbial decontamination, antioxidant activities, and chemical compositions of dried shiitake mushroom powder.

MATERIALS AND METHODS

Materials

Shiitake mushrooms (*Lentinus edodes*) were purchased from Pamieng Royal Project in Chiang Mai Province, Thailand. The mushrooms were dried with a hot air oven (Memmert UFE 600, Schutzart, Germany) at 45 °C for 24 hours. The dried mushrooms were ground into powder by a grinder (Philips). The powder was passed through a 100-mesh sieve (powder size 0.15–0.25 mm). Then 100 g of mushroom powder were packed in aluminum foil bags (size 6 x 9 inches) and were stored at room temperature until use.

Gamma irradiation

Gamma irradiation was carried out in a cobalt-60 Gamma Chamber (GC- 5000, BRIT, India) at Thailand Institute of Nuclear Technology (Nakhon Nayok, Thailand). Shiitake powder samples were exposed to irradiation at a dose of 2.5, 5.0, 7.5 and 10.0 kGy at a dose rate of 3.33 kGy/h at room temperature and normal relative humidity.

Analysis of microbiological properties

The standard plate count method was used to enumerate the total microbial load of the colony forming units per gram (CFU/g) in the control and irradiated samples. The samples were serially diluted and plated separately on plate count agar and potato dextrose agar. Microbiological evaluations of irradiated and non-irradiated shiitake samples were conducted according to the method provided by the Association of Official Analytical Chemists (AOAC, 1990). The initial microflora in shiitake was obtained by homogenizing 25 g of each sample with 225 mL of Butterfield's phosphate buffer using a lab-blender to give 0.1-fold dilution (1:10). Serial dilutions up to 10^{-5} were prepared. For the determination of total viable bacterial count (TVC) and total yeast and mold count (TYM), 1 mL of the appropriate dilution was assayed by a pour-plate method on plate count agar (PCA) and potato dextrose agar (PDA), respectively. All samples were then incubated at 35 °C for 48 h and 25 °C for 5 days, respectively.

Analysis of antioxidant activity

Total phenolic content. The total phenolic content was determined by the Folin–Ciocalteu assay according to the method developed by Velioglu et al. (1998). Phenols react with phosphomolybdic–phosphotungstic components in the Folin–Ciocalteu reagent and produce a blue colored complex which is measured at 725 nm. First, 0.75 mL of 10 fold diluted Folin–Ciocalteu reagent and 100 μ L 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 by using a spectrophotometer (Shimadzu UV 1700, JAPAN). The standard calibration curve was plotted by using gallic acid at the concentrations of 0.02-0.10 mg/mL. The total phenolic content was expressed as mg gallic acid equivalents (GAE)/g sample.

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Determination of free radical scavenging power was performed as previously described by Khattak et al. (2008) with slight modification. Briefly, 100 μ L of each extract was added to 900 μ L of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol solution (150 μ M) and the solution was shaken vigorously. After incubation at room temperature for 15 min in the darkness, the absorbance of each solution was determined at 517 nm by using a spectrophotometer (Shimadzu UV 1700, JAPAN). The free radical scavenging power was expressed as ascorbic acid equivalent (AAE)/g sample.

FRAP (ferric reducing antioxidant power) assay. The FRAP reagent contained 16.7 mM 8.3 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 8.3 mM 2,4,6-tripyridyl-s-triazine (TPTZ) with 250 mM acetate buffer, pH 3.6. A total of 75 μ L sample and 225 μ L of distilled water were added to 2.25 mL of freshly prepared FRAP reagent in a test tube. The mixture was incubated at room temperature throughout the reaction. The absorbance was read at 596 nm using a spectrophotometer immediately and 30 min after mixing. The antioxidant potential of samples was analyzed based on a calibration curve plotted using $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at concentration ranging from 400 to 2,000 μ M.

Analysis of protein content

Determination of crude protein was determined by Kjeldahl method, using Gerhardt equipment (Germany). Dried sample (2.0 g) was digested with 20 ml of 98% H_2SO_4 , using 7 g of the potassium sulfate and 0.7 g of copper sulfate mixture as the catalyst. The digestion was continued for three hours after the digestion mixture turned clear green. Then 80 ml of 40% sodium hydroxide solution was added, and the mixture was distilled for 3 minutes. The distillate was collected in a flask containing 100 ml of 5% boric acid solution, with methylene blue and methyl red as the indicators. The distillate was then titrated with 0.1 N H_2SO_4 solution; the end point was pink. Crude protein was calculated as the percentage on the wet weight basis ($\text{N} \times 6.25$).

Analysis of glucan content

Total and α - glucans contents were determined in the polysaccharide extracts by using the Mushroom and Yeast β -glucan Assay Procedure (Megazyme International Ireland Ltd., Wicklow, Ireland). To evaluate the total glucan content polysaccharide samples were hydrolyzed with 1.5 mL concentrated hydrochloric acid for 45 min at 30 °C. After neutralization, hydrolysis was proceeded into

glucose by using a mixture of exo-1,3- β -D-glucanase (20 u/mL) plus β -glucosidase (4 U/mL) in sodium acetate buffer (pH 5.0) for 1 h at 40 °C. After that, glucose oxidase/peroxidase (GOPOD) reagent was added and incubated for 20 min at 40 °C. The absorbance of all solutions was detected by UV-Vis spectrophotometer at 510 nm. The β -glucan content was calculated by subtraction the α -glucan from the total glucan content. All values of glucan contents were expressed as percent of a sample dry weight.

Statistical analysis

All determinations were conducted in three experiments. All results were calculated as mean \pm SD (standard deviation). Mean values, standard deviation, analysis of variance (ANOVA) were computed by using a commercial statistical package SPSS 21.0 (USA). The data was compared using Duncan's multiple range tests at 5% significance level.

RESULTS

Effect of gamma irradiation on microbial load

Total elimination of the microorganism is the principal aim of food preservation by irradiation. The effects of gamma irradiation at various doses on total viable counts and total yeast and mold are shown in Table 1. It was noticed that gamma radiation caused a reduction in total viable counts. The total viable bacterial counts significantly exceeded total yeast and mold counts in shiitake samples. The total viable counts decreased as the irradiation dose increased. The non-irradiated samples of shiitake were contaminated with bacteria at 1.35×10^3 CFU/g. The values were below the level of 1.0×10^4 CFU/g reported by WHO (1998) as the maximum permissible total count level. The lowest irradiation dose of 2.5 kGy decreased the TVC by 99%. At 5.0 kGy, bacteria were completely eliminated. The counts of yeasts and molds were eradicated after irradiation at 2.5 kGy. Thus, gamma irradiation at 5 kGy was sufficient to practically eliminate contaminated bacteria, yeasts and molds.

Table 1. Effect of gamma irradiation on total viable bacterial counts and total yeast and mold counts of shiitake mushroom powder.

Dose (kGy)	Total viable counts (CFU/g)	Total yeast and mold count (CFU/g)
Non-irradiated	1.35×10^3	<5
2.5	3.0×10^1	ND
5.0	ND	ND
7.5	ND	ND
10.0	ND	ND

Note: ND = No microbe detect on plates.

Effect of gamma irradiation on antioxidant activities

The results of total phenolic content and antioxidant activities of dried shiitake mushroom before and after irradiation are presented in Table 2. The results indicate that gamma irradiation at a dose of 2.5-10 kGy reduced the antioxidant activities in dried shiitake mushrooms. The total phenolic contents in shiitake treated with 10 kGy were significantly ($P \leq 0.05$) lower than in the control. The value of non-irradiated shiitake was 9.66 ± 0.13 mgGAE/g whereas irradiated sample at 10 kGy was 8.15 ± 0.1 mgGAE/g. The total phenolic content of shiitake irradiated was decreased by 6.8%, 6.3%, 15.9% and 15.6% at an irradiation dose of 2.5, 5.0, 7.5 and 10.0 kGy, respectively compared to non-irradiated sample.

Relating to antioxidant activities, DPPH and FRAP assay were applied in this study. The results revealed that there was a significant decrease in the DPPH activity as the irradiation dose increased from 2.5 to 10 kGy as shown in Table 2. The DPPH activity of control was 3.16 ± 0.11 mgAAE/g while the irradiated samples were 2.64 ± 0.12 , 2.45 ± 0.07 , 2.18 ± 0.17 and 2.07 ± 0.02 mgAAE/g, respectively. Similarly, gamma radiation affected antioxidant activity of ferric reducing antioxidant power (FRAP). The FRAP values of non-irradiated and irradiated samples were within the same range 40.24 ± 0.41 – 54.99 ± 2.55 $\mu\text{molFeSO}_4/\text{g}$. The FRAP value of control was 54.99 ± 2.55 $\mu\text{molFeSO}_4/\text{g}$ while the irradiated samples varied in the range of 40.24 ± 0.41 to 48.82 ± 1.40 $\mu\text{molFeSO}_4/\text{g}$. Increasing the gamma radiation dose caused the decrease of DPPH and FRAP values (Table 2). The DPPH and FRAP values of the shiitake mushroom powder reduced significantly by 34% and 27%, respectively when the gamma dose was increased from 0 to 10 kGy. The highest DPPH and FRAP values were observed for non-irradiated shiitake samples. These values were 3.16 ± 0.11 mgAAE/g and 54.99 ± 2.55 $\mu\text{molFeSO}_4/\text{g}$.

Table 2. Analysis of antioxidant activities by total phenolic content, DPPH and FRAP of shiitake mushroom powder at varied irradiation doses.

Irradiation dose (kGy)	Total phenolic content (mgGAE/g)	DPPH (mgAAE/g)	FRAP ($\mu\text{mol FeSO}_4/\text{g}$)
Non-irradiated	$9.66^c \pm 0.13$	$3.16^c \pm 0.11$	$54.99^c \pm 2.55$
2.5	$9.00^b \pm 0.09$	$2.64^b \pm 0.12$	$48.82^b \pm 1.40$
5.0	$9.05^b \pm 0.47$	$2.45^b \pm 0.07$	$45.29^b \pm 0.60$
7.5	$8.12^a \pm 0.48$	$2.18^a \pm 0.17$	$41.36^a \pm 1.60$
10.0	$8.1^a \pm 0.10$	$2.07^a \pm 0.02$	$40.24^a \pm 0.41$

Note: Different letters in the same column represent significant differences ($P \leq 0.05$).

Effects of gamma irradiation on protein content

The protein content of non-irradiated and irradiated samples is shown in Table 3. The results show that there were no significant differences in protein content between non-irradiated and irradiated samples. The protein contents were ranked from 28.45 ± 0.24 to 28.83 ± 0.18 %w/w.

Effects of gamma irradiation on β -D-glucan content

In this study, β -D-glucan content of shiitake mushroom powder was determined by Megazyme method. Gamma irradiation at all the tested doses (2.5, 5.0, 7.5 and 10.0 kGy.) did not significantly affect changes in β -D-glucan content in comparison with non-irradiated samples. The amount of β -D-glucan ranged from 23.27 ± 1.21 to 25.11 ± 0.51 %w/w (Table 3).

Table 3. Total protein content and β -D-glucan content of dried shiitake mushroom powder at varied irradiation doses.

Irradiation dose (kGy)	Total protein content (% w/w)	β -D-glucan content (% w/w)
Non-irradiated	$28.59^a \pm 0.27$	$23.27^a \pm 1.21$
2.5	$28.45^a \pm 0.24$	$24.18^a \pm 1.05$
5.0	$28.54^a \pm 0.12$	$23.86^a \pm 0.93$
7.5	$28.83^a \pm 0.18$	$24.34^a \pm 0.69$
10.0	$28.60^a \pm 0.07$	$25.11^a \pm 0.51$

Note: Different letters in the same column represent significant differences ($P \leq 0.05$).

DISCUSSION

Gamma irradiation as a food preservation technology is a method that is widely used for improving food safety for fresh foods and dried raw materials. It is the best method to eliminate foodborne microorganisms and pests without the nutritional or sensory qualities of foods alteration (WHO, 1998). The results from this study showed that gamma radiation eliminated the total viable and total yeast and molds count at a dose of 5 kGy. A similar result was found in green tea leaf samples (Fanaro et al., 2015) which showed the gamma irradiation at a dose of 5 kGy was the minimum dose to eliminate bacterial and fungal growth. The diminution of total viable count and total yeast and mold count might be due to the direct effect of radiation as well as the indirect effect from radiolysis. The effect of radiation on microorganisms was attributed to ionizing irradiation acting directly or indirectly on DNA and inducing direct modifications of molecules. In addition, the radiolysis which was the interaction of ionizing irradiation with water molecules caused free radicals formation. These products had a very unstable structure that had strong reactivity with the nearby biological

molecule (water, protein, DNA). Their interactions with DNA produced chemical modifications of polymer such as oxidation (IAEA, 2003). The indirect effect of radiation was responsible for 70% of all radiation effects (Diehl, 2002). Furthermore, increasing the irradiation dose achieved the decontamination effect.

The total phenolic content of control and irradiated dried shiitake was determined by using Folin-Ciocalteu's reagent. The results were expressed as mg equivalents of gallic acid/g of sample. Generally, gamma irradiation significantly either decreased or increased the total phenolic contents. For shiitake powder, gamma irradiation at doses from 2.5 to 10.0 kGy significantly influenced to phenolic contents in comparison with non-irradiated sample. The increase in irradiation dose caused a decrease of the phenolic content. This result was consistent with Ahn et al., (2005) who observed a significant reduction on the total phenolic content of Chinese cabbage (*Brassica rapa* L.) at dose over 1 kGy. Villavicencio et al., (2000) also revealed that the total phenolic content in Macarca bean was significantly reduced by gamma radiation at 10 kGy. This consistent with Zhu et al. (2010) who reported a significant decrease in total phenolic acid contents of black rice at a dose of 2, 4 and 6 kGy gamma irradiation. Similarly, Koseki et al. (2002) reported a decline in the amount of total phenolic compounds in dehydrated rosemary after irradiation at a dose higher than 10 kGy. The apparent decrease of total phenolic content may be due to a disruption of phenolic acid by gamma radiation (Zhu et al., 2010).

The antioxidant activities of dried shiitake mushroom were determined by scavenging DPPH radical and FRAP assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable radical deep purple in color that is used to evaluate the ability of an antioxidant for providing proton and its color changes to yellow in the presence of an antioxidant (Gadow et al., 1997). The inhibition activity of the non-irradiated and irradiated solution samples were examined for their radical-scavenging activity. The DPPH values of irradiated shiitake were lower when compared to non-irradiated sample. Likewise FRAP assay, the FRAP values of irradiated samples were lower when compared to non-irradiated sample. The results are consistent with Lampart et al. (2003) who found that the increase of irradiation dose to 10 kGy caused a decrease of antioxidant activities in lupin seed product. This result differed from Aouidi et al. (2011), who reported the absence of a significant effect of gamma irradiation at 20 kGy on antioxidant activity of air-dried olive leaves. Lee et al. (2005) also revealed that gamma irradiation up to 25 kGy did not have any effect on the antioxidant activity of green tea byproducts and green tea leaf extract. Meanwhile, Pewlong et al. (2016) reported an increase of glycyrrhizic acid in licorice powder for gamma irradiation at a dose 15 kGy.

The effects of gamma irradiation on chemical compounds including total protein content and β -D-glucan content were investigated in this study. The results showed that no significance in protein and β -D-glucan content when compared

to non-irradiated sample. The results were similar to Byun et al. (1996) and Stajner et al. (2007), who reported that gamma irradiation at doses up to 10 kGy did not induce significant loss in water soluble components such as minerals, nitrogenous constituents, sugars and proteins in soybean seeds. Relating to protein conformation, gamma radiation at a dose as high as 50 kGy did not significantly alter protein quality (Eggum, 1979). Beta glucans are polysaccharides of D-glucose monomers linked by β -glycosidic bonds. β -glucans are contained in many mushrooms such as *Ganoderma lucidum*, *Grifola frondosa*, *Pleurotus abalones*, *Flammulina velutiper* and *Auricularia auricular*. Shiitake (*Lentinus edodes*) mushroom is the most important sources of β -glucan (Zhu et al., 2015). The increased scavenging activity of β -glucan after irradiation could be due to the fragmentation of the polysaccharide chain to low molecular weight subunits that leads to the exposure of their hydroxyl groups and decreasing the intramolecular hydrogen bonding (Xing et al., 2005). Therefore, the shiitake β -glucan was not damaged by gamma radiation at 10 kGy.

CONCLUSION

The gamma radiation affected microbial load and chemical properties. Increasing gamma irradiation doses resulted in decreases on microbial load, antioxidant activities (total phenolic content, DPPH and FRAP), while total protein content and β -D-glucan content were not altered. The gamma radiation at a dose 5.0 kGy was sufficient for microbial decontamination without total protein and β -D-glucan content variation.

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